STABILITY AND RHEOLOGICAL BEHAVIOUR OF FUNCTIONAL ESSENTIAL OILS IN GLYCOLIPID CREAM EMULSION

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FACULTY OF SCIENCE
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ABSTRACT

Essential oils carries distinctively unique aroma from parts of a plant where they are extracted. Essential oils have widely been known for its aromatherapy and therapeutic effect on human. High concentration of essential oil comprises of thyme, lavender, tea tree and citronella oil have been induced in emulsions system using olive oil as carrier oil stabilized by glycolipid surfactants of sucrose stearate, surcrose palmitate and sucrose myristate respectively. Co-surfactants, Triton^{TM} X-100 and Triton^{TM} X-45 was utilised to create high interactions and compatibility associated with short chain terpenes, terpenoids and phenolic compound presents in essential oils inverting emulsion droplet forming o/w emulsion system. Essential oil emulsions were subjected to a series of physical properties evaluation specifically accelerated ageing test conducted at 45.0±0.1 °C, zeta potential, mean droplet size measurement and polarising microscope observation. Rheological evaluations by mean of oscillatory and viscometric studies were carried out at constant temperature of 30.0±0.1 °C over the period of 30 days. Viscometric flow curve shows shear thinning behaviour for all essential oil emulsions exhibiting reduction of yield stress directly proportionate with reduction of lipid chain length. Mean droplet size and PDI govern the variation of viscosity of thyme and lavender oil where large droplet forming uniform distribution yield high viscosity emulsion possessing strong structural integrity with high critical strains and *G'* magnitude. Frequency dependent shear oscillation display dominancy of *G'* over *G"* throughout the measured frequency (ω) domain implicates emulsions system exhibiting solid like behaviour. Thyme and lavender essential oil emulsions possessing higher *G'* and viscosity was concluded to be more stable than tea tree oil and citronella emulsions. All the emulsion tested possessed a long shelf life sustaining high emulsion fractions over 30 days of storage.

ABSTRAK

Minyak pati mempunyai aroma unik yang tersendiri dimana pati tumbuhan diekstrak daripada bahagian tumbuhan seperti pucuk, daun, dan pohon. Minyak pati digunakan secara meluas untuk tujuan aromaterapi untuk memberi kesan terapeutik ke atas pemakainya. Miyak jenis 'thyme', 'lavender', 'tea tree' dan 'citronella' telah digunakan dalam kepekatan tinggi dalam pembentukan system emulsi yang distabilkan oleh miyak zaitun dan surfaktan glycolipid seperti stearat sukrosa, palmitat sukrosa dan myristat sukrosa. Triton™ X-100 dan Triton™ X-45 digunakan untuk meningkatkan interaksi dan keserasian pembentukan dengan hidro-karbon rangkaian pendek dan sebatian fenolik yang wujud dalam miyak pati untuk membentuk sistem emulsi o/w. Penilaian kestabilan emulsi miyak pati telah dijalankan dengan mengunakan beberapa kaedah ujian seperti pengukuran kadar penceraian emulsi pada suhu 45.0±0.1 °C, potensi zeta, min saiz titisan yang terbentuk dan permerhatian fizikal emulsi melalui mikroskop polaroid. Penilaian reologi berdasarkan pengajian osilatori dan 'viscometric' telah dijalankan pada suhu malar 30.0±0.1 °C dalam tempoh 30 hari. Data 'viscometric' memaparkan pengurangan daya kelikatan emulsi dan tegasan ricih yang berkadar terus dengan pengurangan kepanjangan rangkaian lipid. Taburan dan min saiz titisan emulsi memainkan peranan penting dalam mengawal kelikatan emulsi, pembentukan saiz titisan emulsi yang besar dan tersusun meningkatkan daya geseran diantara titisan emulsi serta terbinanya struktur integriti yang kukuh dengan terikan kritikal yang tinggi selaras dengan peningkatan magnitud tenaga *G'* menjurus kepada peningkatan daya kelikatan emulsi dan keseluruhannya menstabilkan emulsi. Pengajian osilatori frekuensi memaparkan dominasi *G'* kepada *G"* menunjukkan ciri-ciri pepejal sistem emulsi. Kesimpulannya, emulsi 'thyme' dan 'lavender' yang memiliki tenaga *G'* dan daya kelikatan yang lebih tinggi adalah lebih stabil daripada emulsi 'tea tree' dan 'citronella'.

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbols

ω	Angular Speed
ηa	Apparent Viscosity
A	Area
V_A	Attraction Energy
κ_B	Boltzmann Constant
E_c	Cohesive Force
V_T	Combined Energy
η^*	Complex Viscosity
β	Cone Angle
ε	Dielectric Constant
D	Displacement
η	Dynamic Viscosity
U_E	Electrophoretic Mobility
σ	Extensional Stress
ρ	Fluid Density
F	Force
f	Frequency
g	Gravity
H	Height
$f(\kappa a)$	Henry's Function
γ_I	Interfacial Tension

Symbols

ν	Kinematic Viscosity
M	Median
W_{min}	Minimum Work
μ	Newtonian Viscosity
a	Parcel Radius
φ	Porosity
R	Radius
V_R	Repulsion Energy
γ	Shear Rate
τ	Shear Stress
η	Viscosity
ρs	Solid Density
$\dot{\gamma}$	Strain Rate
T	Temperature
h	Variable Plate Gap
V	Velocity
Φ	Volume Fraction
τ^0	Yield Stress
ζ	Zeta Potential

Abbreviations

Ce-20	Ceteareth-20
CMC	Critical Micelle Concentration
CP	Cone and Plate
cP	Centipoise
cSt.	Centistokes
DLS	Dynamic Light Scattering
GC-MS	Gas Chromatography-Mass Spectrometry
i.d.	Internal Diameter
LDL	Low-Density Lipoprotein
LDV	Laser Doppler Velocimetry
LVR	Linear Viscoelastic Region
PDI	Polydispersity Index
PIT	Phase Inversion Temperature
PP	Plate and Plate
RO	Reverse Osmosis
rpm	Revolutions Per Minutes
SAD	Surfactant Affinity Difference
S.I.	International System
SM	Sucrose Myristate
SP	Sucrose Palmitate
SS	Sucrose Stearate
TX	Triton™
Wt%	Weight Percentage

Abbreviations

O/W	Oil in Water
O/W/O	Oil-in-Water-in-Oil
W/O	Water in Oil
W/O/W	Water-in-Oil-in-Water

LITERATURE REVIEW

Cosmetic industry is a fast growing industry that evolves rapidly to fulfil the demand of human on maintaining and improving physical outlook from using simple application of cosmetic products on skin surface to the highly complicated corrective surgery to achieve satisfactory results [1, 2]. Safety of cosmetic products has been a debate for a long time. Increasing awareness and education in the society has changed the direction of the cosmetic industries, as more users are optioning for product produced from natural ingredient as compared to synthetic chemical products.

Cosmetic products are normally being applied topically on skin and leaves skin with a protective barrier while certain active ingredient penetrates into the skins and gets into the body system [1, 3]. Semisolid emulsions, oil-free dispersions, lotion and fluid are used as vehicle to deliver active ingredient such as drugs and cosmetic agents to the skin as these mobile dispersions enhance the therapeutic of properties topically applied products on skin [4-7].

Aroma plays a key role in attracting consumers with a pleasant odour and increases the interest of consumers in products purchasing. Aroma from fragrances is usually added into cosmetic creams and cosmetic toiletries, but the use of synthetic fragrance sometimes causes irritation or triggers allergic reaction to users with sensitive skin [7, 8]. Therefore, natural essential oil has been adapted in cosmetic industry nowadays as a substitute to fragrances.

Apart from having characteristic odour, essential oil also possesses beneficial properties such as antimicrobial, antibacterial, antioxidant, anti-ageing and others resulting from the chemical compound present. Increased in demand and popularity of essential oil resulted in increased adaptation of essential oil in cosmetic toiletries as

additives for product such as soap, fragrances, creams and lotions in replacing synthetic fragrances [8].

Careful selection and understanding of essential oil had to be made before selecting and using the desired essential oil. Like other chemicals, essential oils were graded into a few categories where some would cause harm when used. Pure grade essential oils are safe to be used even in high amount with the condition that they have been toxicologically certified safe to be used [9, 10]. Some essential oil such as buchu, mugwood, bitter fennel, star anise, thuja, wormwood and wintergreen are not recommended for use due to the presence of highly toxic compounds in them [8-10].

High production cost of pure essential oil causes industry to substitute or to add synthetic chemical compound to refined essential oil as a substitute to the naturally occurring compound in essential oil. Addition of synthetically produced chemical compound reduces the price of essential oil giving consumers a choice of purchasing essential oil at a lower price. Synthetic essential oil is not suitable to be applied directly on skin as they would most likely to cause sensitivity to users if used in high dose [10]. Therefore, the potential use of high dose essential oil has been hindered due to safety and suitability of oil used.

Although essential oil has been well known for its beneficial property, its potential has been hindered to a minute amount usage due to the presence of corrosive and toxic compounds in some essential oil and its instability in forming emulsion if used in high concentration [10]. In this project, emulsion containing high composition of pure essential oil was formulated and studied to increase the potential application of essential oil in the future.

Essential oil such as thyme oil, lavender oil, tea tree oil and citronella oil have been selected as a subject of study. Food grade natural vegetable oil which is olive oil has been used as carrier oil and diluent of essential oil where high amount of essential oil can be introduced into the emulsion system with higher probability of formation. Food grade olive oil is tested to be safe for consumption [11, 12]. Therefore, ingesting emulsion produced from this carrier oil would not cause harm to human. Glycolipid surfactants used in the formulation are of food grade material where they are safe for consumption in a controlled quantity. As natural ingredients are preferred in the industries today, selection of chemicals used must be of natural and least harmful to human.

CHAPTER 1: INTRODUCTION

1.1 Essential Oil

Essential oil, an essence of a plant which carries distinctive aroma which is ‘essential’ as their name suggested. Essential oil comprise of mixtures of chemical compounds such as terpenes, terpenoids, alcohols, aldehydes and phenols which bring about the beneficial properties such as antibacterial and anti-fungi property which acts as the defensive system of a plant against harmful environment and organism [9, 10]. Apart from that, they also possesses properties such as antioxidant, anti-ageing, expectorant, carminative and other beneficial properties acting synergistically to give therapeutic effects [10]. Every essential oil is made up of chemical components which are distinctively different from each other which bring about the difference in the aroma and the function each carries. Most essential oils extracted are biologically active [13].

As the pleasant aroma would soothe and relax human mind and body, essential oil has been widely used in aromatherapy sector as fragrant oil and massage oil. Essential oil is highly volatile hence evaporates easily in air [10]. Inhaling essential oil would help to calm and relax exhausted mind and relax cramp muscle which developed from daily activities while massaging diluted essential oil or cream containing essential oil would assist the absorption of the essential oil into the skin layer [14, 15].

Essential oil mostly takes effect on the limbic system of human brain by stimulating olfactory bulb which has close anatomical ties to the limbic system, responsible for emotional expressions of a person [14]. Aroma from the essential oil sends signal to the olfactory bulb where then the limbic system would influence the endocrine and autonomic nerves of the body [15]. Absorption of essential oil through the skin was an alternative route where small molecules of essential oil permeate through the skin barrier into the human blood system and exert a measurable psychological effect [14, 15].

Extraction of essential oil was usually carried out by using hydrodistillation and steam distillation method [16]. These methods are usually being adopted due to their simplicity in handling and cleaner as compared to solvent extraction method which usually consumes a great amount of solvent.

Essential oil also shows important applications in culinary sector as they had been used in culinary in European countries as a taste enhancer and as food preservatives. Development of technology and lifestyle evolvement does not change the habit of natives using essential oil in food. This shows the wide applications of essential oil and their persistency in the industry [17, 18].

Replacement of synthetically produced and harmful material has shown importance in cosmetic industries as synthetically produced cosmetic ingredient sometimes would cause skin sensitization and may also trigger allergic reaction. The use of synthetically produced preservatives such as parabens might be reduced by adding essential oil as a component of the formulation as the oil possesses antibacterial and antimicrobial properties which are responsible for the reducing of the degradation of cosmetic product by these microorganism [18].

Generally, paraben had been used as preservatives to maintain product integrity in cosmetic industries because they are cheap and can be easily obtained. Study on antimicrobial properties shows that essential oil such as thyme, lavender, tea tree, cinnamon, eucalyptus and sandalwood has high potential to be natural preservatives which can serve as replacement for harmful material such as parabens [18, 19]. Apart from its beneficial properties, essential oil also carries pleasant aroma which would counter the need of use of fragrance in cosmetic.

1.1.1 Thyme Oil

Thyme which originated from *Labiatae* family is a small subshrub, low-growing perennial herbaceous plant which has been used since ancient time as medicinal herbs and as a culinary. It is cultivated and harvested in countries such as in Spain, Portugal, France, Greece and India [16]. There is a large variety of thyme that could be found for example lemon thyme, garden thyme and silver thyme with variation of scent and application. The chemical component present varied with geographical origin of production and species of thyme cultivated [16, 20].

Thyme takes its name from Greek word "thymos" which means "to perfume". Thyme has long been used by ancient Egyptians for embalming, ancient Greeks in their baths and ancient Romanians in purifying their room [20]. Thyme herbs has been ingested and added into food as preservative and seasoning since ancient times and this culture has remained until today. Fresh or dried thyme leaves are added into cooking as taste enhancer while it naturally preserves the food quality [21].

Thyme has a very strong, sharp and persistent aroma which is mainly due to the presence of thymol and carvacrol [16]. Extracts from Thymus vulgaris yield two types of thyme essential oil where the first extraction produces the crude extract which is red thyme and white thyme or sweet thyme which is produced from subsequent extraction of red thyme [22]. Red thyme consists of the major constituent of the thyme extracts of thymol and other phenolic monoterpenes. It possesses a sharp spicy aroma while white thyme is a milder type of essential oil produced and possesses a softer sweet smell [23]. White thyme can be applied to elderly people and even children while red thyme cannot be applied to both categories due to the high content of phenolic compound [19].

Due to the presence of phenolic monoterpenes, thyme oil shows high resistance to microbial activity [19, 22]. They are highly antiseptic, antimicrobial and anti-inflammatory therefore, they have been highly used as disinfectant in hospitals and as

an active ingredient in antiseptic creams. Thyme has also been used in cough syrup to act as carminative, expectorant and spasmolytic agent to treat diseases related to bronchi [21]. Besides that, properties such as antioxidant, anti-inflammatory and anti-ageing it possesses meets the requirement of cosmetic applications in preserving the youthfulness of skin, promoting the health and hygiene of skin [9, 24-28].

Figure 1.1: Image of fresh thyme herbs used for culinary purposes.

1.1.2 Lavender Oil

Lavender oil extracted from plants of genus *Lavandula* comprises of many different species belonging to *Lamiaceae* family, namely *Lavendula officinalis*, *Lavendula angustifolia* and *Lavendula vera*. The name lavender was derived from its Latin name ‘Lavare’ which means ‘to wash’. These plants were grown in Mediterranean countries, Middle East, China and India [29] and had been widely used for their pharmacological properties in medical purposes [30-33]. Essential oil extracted from these plants consisting mainly mixtures of terpenes and terpenoid [34, 35].

Uses of lavender oil dated back to 2,500 years ago, where the beautifully scented lavender has been widely used in perfume making industries. Modern aromatherapy application was introduced when a chemist and perfumer Rene-Maurice Gattefosse (1881-1950) accidentally plunged his burned hand into neat lavender oil and

discovering that his wound healed rapidly without scarring. The discovery of medical application of essential oil has redirected the research of essential oil, not limiting merely to perfumery but also extending to development of complementary-alternative medicinal applications [10].

Lavender oil has been used as an antibacterial, antifungal, sedative, anti-depressive agent and works effectively for burns and insect bites [36]. It blends readily with other essential oils such as cedar wood, pine, geranium, nutmeg, thyme and ylang ylang to give a pleasant smell and to improve therapeutic effect of essential oil acting synergistically.

Lavender oil serves an important role in the fragrance industries due to its delightful odour and its psychological effects on human. Increase in relaxation, mood improvement, sedation [36], anti-conflict [37], and altered motor activities [38] are shown when exposed to lavender oil. In addition, it also increases the human concentration and accuracy in completing a task [39]. Several products such as soaps, colognes, perfumes, skin lotions and other product had been produced by using this essential oil [40, 41]. Lavender oil also has been used in food manufacturing industry as beverages, ice creams, candy and confectionaries flavouring [42].

Figure 1.2: Image of contrived lavender plant.

1.1.3 Tea Tree Oil

Melaleuca alternifolia with the common name of tea tree is a native plant in Australia categorized in the *Myrtaceae* family. It has a feature of tree with needle like leaves. Early discovery of uses of tea tree are highly linked to aborigines of Australia which uses it to treat ailment from wound treatment to cold and cough treatment [43]. Tea tree is further introduced by Captain James Cook which landed nearby Sydney, Australia (1770), who later discovers the uses, benefits and medicinal value of tea tree from natives of Australia after noticing that the land was filled with massive groves of trees with thick and aromatic leaves. Thereafter, there was widespread use of tea tree oil among Australian settlers in the 19th century and until today. Tea tree oil has still been extensively used for its therapeutic properties [43-45].

Tea tree has been widely cultivated for commercial purposes in the coastal region of northen New South Wales and the Atherton Tablelands of Queensland . Tea tree oil extracted from *Melaleuca alternifolia* consist mostly terpenes hydrocarbon and tertiary alcohols. The main component of tea tree comprises of terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, *p*-cymene, α-terpineol, α-pinene, terpinolenes, limonene and sabinene [43-45]. Researcher found that tea tree act actively in antimicrobial activity and it treats cutaneous skin infections effectively [47-49]. Therefore, they are used broadly for treating acne and skin related disease. Apart from that, it has also been used to treat cold sores, dandruff, onychomycosis, oral candidiasis and tinea pedis by topically applied to the skin surface [50].

Tea tree oil is only safe for topical applications and not for consumption, unlike thyme oil, lavender oil and citronella oil which can be consumed and be added as one of the taste enhancer in food [50, 51]. Presence of 1,8-cineole, a skin irritant in tea tree oil might induced skin sensitization and irritation if used at a high amount [48]. Tea tree oil would cause intoxication if consumed at a certain high amount supported by the

evidence from experiments studied on human and rat [51, 52]. Clinical studies reveal that ingestion of tea tree oil would cause central nervous system depression causing ataxia, unconsciousness and unresponsiveness depending on the amount of ingestion [53]. Therefore, tea tree oil is relatively safe for low concentration of topical application while not safe to be consumed or used at high dosages [52, 53].

1.1.4 Citronella Oil

Cymbopogon plant is a low growing perennial grass famous for its delightful lemon like aroma used majorly to ward off insects and mosquito [54]. Fresh plant of citronella has been traditionally used in culinary as taste enhancer and as folk remedies for medication purposes (Figure 1.3). The lemon like aroma is due to the presence of limonene which is identical to those found in lemon extracts [54, 55]. Citronella essential oil extracted from *Cymbopogon* genus is graded according to the species of plant and composition of citronellol and citronellal. Java citronella extracted from *Cymbopogon winterianus* contain 16% citronellol and 33% citronellal is graded to be superior than Ceylon citronella extracted from *Cymbopogon nardus* which contain 8% citronellol and 5% citronellal [56]. Citronellal oil comprises fractions of citronellal, geraniol, citronellol, citronellyl acetate, *β*-bourbonene, geranyl acetate, elemol, *L*-borneol and nerol [56-60].

Citronella is widely distributed in the Mediterranean area [54] and South East Asia country especially Ceylon and Java. Taiwan, Sri Lanka, India and Mexico are few countries that contribute to world supply while China and Indonesia are the main producers of citronella oil [61]. Citronella is cultivated for its fresh fragrance leaves which are commonly used in preparation of food and beverages. Citronella shows medicinal properties like bringing back appetite, diuretic and its aqueous extract cures

intestinal troubles, food poisoning and helps digestion [55]. Citronella also can be used to alleviate neurodegenerative disorder [61], treat cough, elephantiasis, malaria, pneumonia and vascular disorders. Research found that citronellal holds antidepressant, antioxidant, antiseptic, fungicidal, astringent, antibacterial, sedative and nervine properties [62].

Figure 1.3: Image of citronella plant captured in Spice Garden Penang, Malaysia.

Table 1.1: Applications of essential oil in industries [9, 24-26, 40, 50, 62].

No	Essential Oil	Applications
1.	Thyme oil	Antiseptic, preservatives, expectorant
2.	Lavender oil	Fragrance, antioxidant, aromatherapy
3.	Tea Tree oil	Antiseptic, antioxidant, acne treatment
4.	Citronella oil	Insect repellent, lice treatment

1.1.5 Carrier Oil: Olive Oil

Carrier oil is a diluent used to dissolve, reducing concentration of active material and as a vehicle to aid absorption of essential oil through skin. Due to the corrosive nature of some essential oil for example thyme oil, diluent must be added to reduce the concentration of phenolic compound present before applying on skin [10]. Differences between essential oil and carrier oil are essential oil comprises of volatile aromatic chemical compounds where they would evaporate easily, would be oxidized but will not turn rancid.

While carrier oil, a vegetable oil comprises of triglycerides, free fatty acids and fat soluble vitamins would turn rancid if kept for a long period of time [63]. Blending essential oil with carrier oil prolong the shelf lives of oil while carrier oil contains liposoluble vitamins and minerals further enhances the therapeutic effect and benefits of essential oil delivered through topical applications [10]. Carrier oils are selected from a range of vegetable oils such as olive oil, grape seed oil, avocado, sweet almonds, jojoba oil and sunflower oil [63].

Table 1.2: Fatty acids composition of different type of vegetable oils [64-66].

Types of Oil	Saturated (mol/mol)	Monounsaturated (mol/mol)	Polyunsaturated Omega-6 (mol/mol)	Polyunsaturated Omega-3 (mol/mol)
Peanut	17-21	40-70	13-28	-
Olive	8-14	65-83	6-15	0.2-1.5
Maize	12-28	32-35	40-62	0.1-0.5
Soya	10-18	18-30	35-52	6.5-9
Sunflower	5-13	21-35	56-66	-

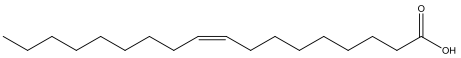
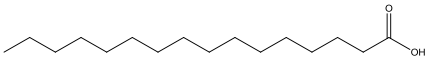
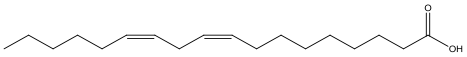
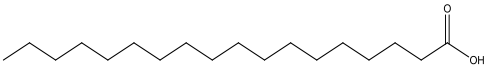
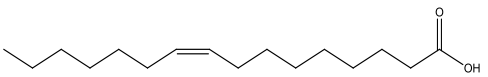
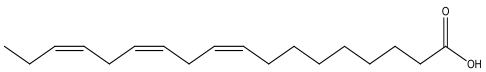
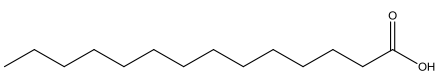
Olive oil served as a staple food for most of the countries in Mediterranean mostly Italy, Spain and Greece [64]. Olive oil contains 98% to 99% triglycerides with 1% to 2% of minor component exerting important values associated with antioxidant power compared to other vegetables oils (Table 1.2 & Table 1.3). Oleic acid present abundantly in olive oil as monounsaturated fatty acids act as skin softener agent in wrinkle prevention [65]. Minor components of olive oil comprises of phenolic compounds (hydroxytyrosol, oleuropeine, caffeic acid, vanillic acid and ferulic acid), carotenoids (β-carotene and lutein), α-tocopherol, squalene, phytosterols, and chlorophyll [66].

Polyunsaturated ω-6 and ω-3 (eicosapetaenoic and docosahexaenoic acids) acts as local anti-inflammatory and immune-protection against infections such as atopic dermatitis, acne, eczema, psoriasis and keep skin hydrated by maintaining skin homeostasis [66, 67]. Squalene, a biochemical precursor of steroids found in olive oil is a potential chemo-preventive compound that has anti-neoplastic effect which inhibits the development and spread of neoplastic cells on colon, breast and prostate [67, 68]. These components hold the ability of olive oil in decreasing LDL cholesterol level, reduces blood pressure, reduce risk of heart disease and tumour, protection from colon and breast cancer [69-72].

Numerous antioxidants present in olive oil act synergistically as derma-protection against atmospheric pollution and solar ray typically ultraviolet ray which causes favouring of cell photo-ageing, lipid peroxidation and development of skin cancer [73-77]. There is an increasing trend where olive oil has been used as key ingredient in soap making, creams, lotions and other products such as hair shampoos and conditioners [78].

Figure 1.4: Image of olive leave captured in Secret Garden Bandar Utama Selangor, Malaysia.

Table 1.3: Composition and types of fatty acid present in olive oil represented in form of methyl ester [79].

IUPAC Name	Common Name	Composition Percentage (mol/mol)	Chemical Structure
Octadec-9-enoic acid	Oleic acid	56.0 – 83.0	
Hexadecanoic acid	Palmitic acid	7.5 – 20.0	
9,12-octadecadienoic acid	Linoleic acid	3.5 – 20.0	
Octadecanoic acid	Stearic acid	0.5 – 3.5	
Hexadec-9-enoic acid	Palmitoleic acid	0.3 – 3.5	
9,12,15-octadecatrienoic acid	Linolenic acid	0.0 – 1.5	
Tetradecanoic acid	Myristic acid	0.0 – 0.5	
Others	-	Minor	

1.2 Surfactant

Surface-active agent termed surfactant is a substance having ability to adsorb at the interface when present at low concentration to form colloidal aggregates altering the interfacial free energies of interfaces (boundary between any two immiscible phases), hence reduces the interfacial tension between two phases [80-82]. Interfacial tension between two phases is measured by determining the interfacial free energy per unit area which is the minimum amount of work (W_{min}) required to create or expand an unit area of interface; $W_{min} = \gamma_I \times \Delta$ *interfacial area*. Therefore, when two surfaces with great dissimilarity meet, the greater the interfacial tension γ_I at the boundary between two phases [82].

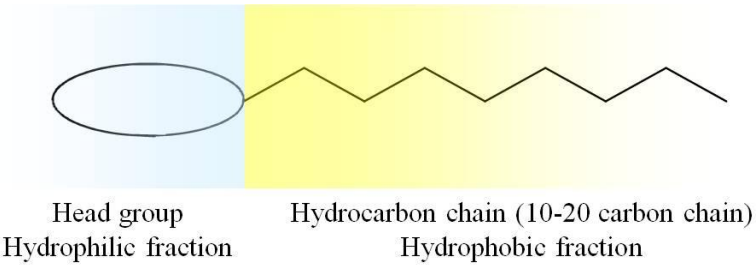


Figure 1.5: Schematic diagram of a surface active molecule.

Surfactant is an amphiphile having a characteristic molecular structure build up of lyophobic (hydrophobic) hydrocarbon tail having preference to organic solvent and lyophilic (hydrophilic) polar head group which has a strong attraction to aqueous solvent (Figure 1.5) [81-83]. Amphiphatic character of surfactant reduces surface tension dramatically at low concentrations [83]. Lyophobic group distorts the orientation of solvent to minimize contact between lyophobic group with solvent increasing the free energy of the system minimizing unfavourable solvophobic (solvent

revulsion) interactions [82-84] enabling surfactant to dissolve favourably in both organic and aqueous solvent.

Interfacial processes such as foaming, detergency and emulsification depends on the concentration, orientation and packing of surfactant at the interface. Interfacial area occupied by a surfactant molecule greatly influenced the effectiveness of adsorption of surfactant at the interface [84-86]. Structural orientation and surfactant head group determine the effective cross-sectional area at interface of surfactant where the smaller the effective cross-sectional area of surfactant, the smaller the interfacial area and hence the greater the effectiveness of adsorption of surfactant at the interface [81-84]. High speed wetting and spreading determines the rate of adsorption which determines the performance of surfactant [82-89].

Surfactant plays a major role in the pharmaceutical and cosmetics industries. It is widely used to stabilize droplet of dispersed phase of emulsion system preventing the separation of two immiscible phases such as oil and water [84]. Surfactant plays a vital role in many applications and products including detergent, paint, cosmetics, pharmaceutical fibres, synthesizing polymer and agrochemical for herbicides and insecticides [83]. Surfactant has been classified according to the formal charge on surfactants head groups namely anionic, cationic, non-ionic and zwitterionic surfactant [88].

(a) Anionic surfactant

The surface-active polar head group bears a negative charge, for example $RCOO^-Na^+$ (soap) and $RC_6H_4SO_3^-Na^+$ (alkylbenzene sulfonate). Anionic surfactants are the largest class of surfactant and generally not compatible with cationic surfactant [83, 84].

(b) Cationic surfactant

Cationic surfactant is a molecule with positive charged head groups, for example primary amine R-NH_2, which would ionize to NH_3^+ ions when dissolve in acid solutions, $RNH_3^+Cl^-$ (salt of a long chain amine) and $RN(CH_3)_3^+Cl^-$ (quaternary ammonium chloride). The most common cationic surfactants used are quaternary ammonium compound which is less sensitive to pH change [82-88].

(c) Zwitterionic surfactant

Zwitterionic surfactant is an amphoteric molecule with both positive and negative charges present on the surface-active portion. For example $RN^+H_2CH_2COO^-$ (long chain amino acid) and $RN^+(CH_3)_2(CH_2)_2SO_3^-$ (sulfobetaine). Zwitterionic surfactant is the smallest groups of surfactant and has the highest compatibility with other classes of surfactants [82-89].

(d) Nonionic surfactant

Nonionic surfactants bear no apparent ionic charge on the surface-active portion. For example $RCOOCH_2CHOHCH_2OH$ (monoglyceride of long-chain fatty acid) and $RC_6H_4(OC_2H_4)_xOH$ (polyoxyethylenated alkylphenol). Nonionic surfactants are prepared by addition of ethylene oxide to long chain hydrocarbons with terminal polar groups like –OH, -COOH. Introduction of ethoxy groups which are polar in nature to form hydrogen bond with water increases the solubility of non-ionic surfactant in water. However, the resulting molecule still possesses amphiphilic character for micelle formation. Nonionic surfactant has lower CMC (critical micelle concentration) value compared to surfactant from other classes. Sensitivity to temperature changes is a distinctive feature of non-ionic surfactant [84-88].

1.2.1 Glycolipid Surfactant

Use of surfactant causes an impact on environment where the biodegradability and toxicity to marine organism became an issue of concern [89, 90]. Therefore, renewable and readily biodegradable surfactant has been opted to replace commercial surfactant in order to favour environmental sustainability and to reduce toxicity impact on marine life. Such surfactant comprises of naturally occurring carbohydrates and amino acids for hydrophilic head groups and natural fats for hydrophobic groups [91].

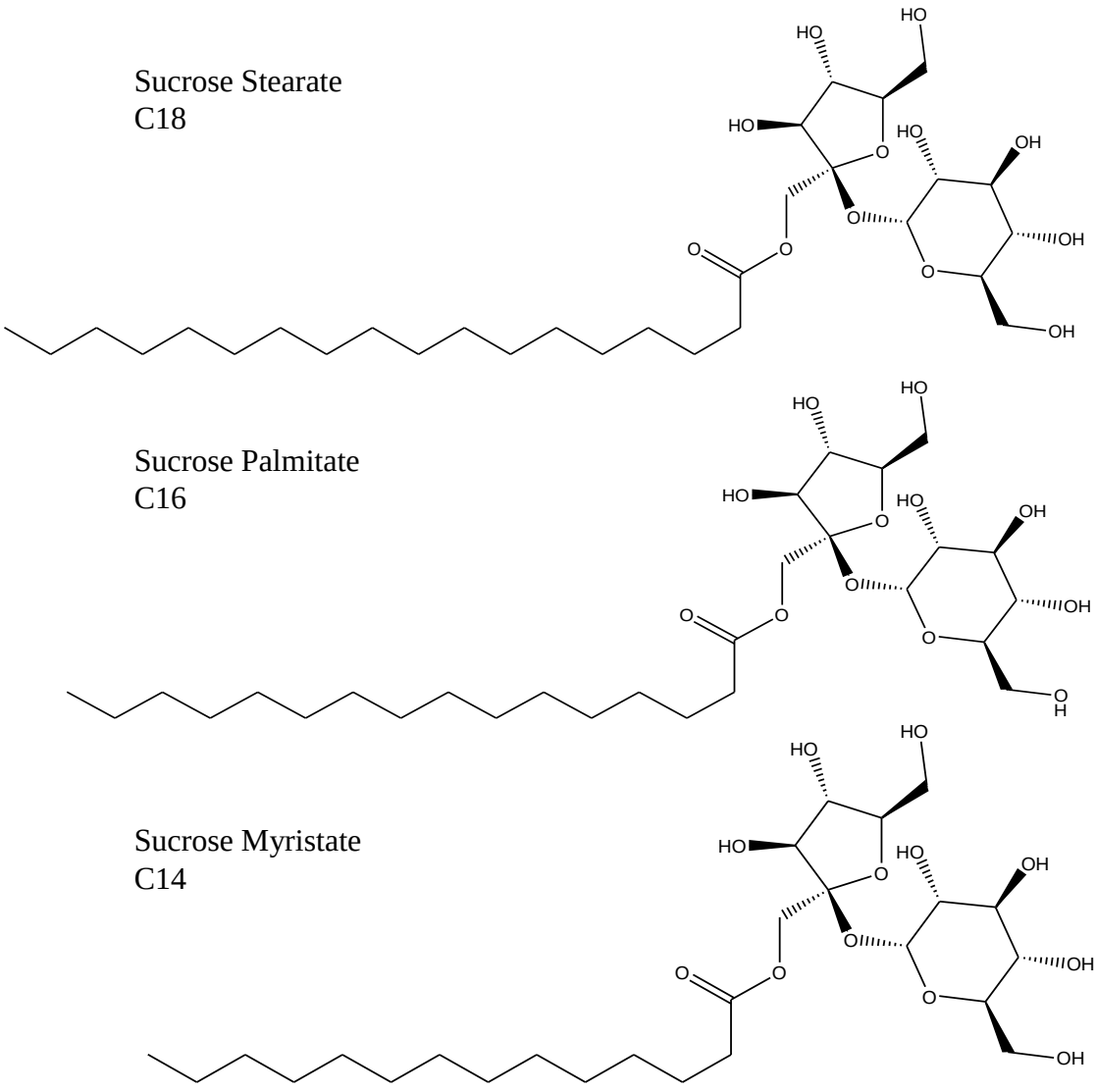


Figure 1.6: The structure of glycolipid surfactant of sucrose stearate, sucrose palmitate and sucrose myristate.

Glycolipid surfactants are biosurfactant comprising of a hydrophobic lipid tail and hydrophilic sugar head group (Figure1.6). These properties enable the surfactant to form a highly organized structure through self-assembly [92]. Glycolipid surfactant such as sucrose stearate (SS), sucrose palmitate (SP) and sucrose myristate (SM) has been used in the formulation of essential oil emulsion. These glycolipid surfactants are made up of a saccharides head groups and hydrocarbon tail or lipid tail which would dissolve in oil. The glycolipids property aid reduction in surface tension and promotes mixing in the emulsion system. Glycolipids are generally nontoxic and biodegradable [93]. With increasing environmental awareness, bio-based surfactants which are biodegradable and safe are preferably selected compared to petroleum based synthetic surfactants [94].

1.2.2 Co-Surfactant: Triton™ X-100 and Triton™ X-45

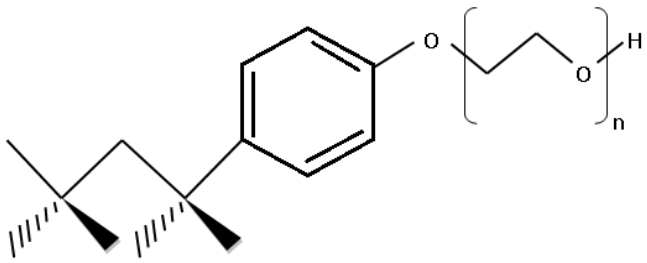


Figure 1.7: Chemical structure of Triton™ X series surfactant.

Triton™ X-100 is a nonionic surfactant consisting of hydrophilic polyethylene oxide group and hydrophobic phenyl group [95]. Triton™ is an octylphenol ethoxylate with high compatibility with other anionic surfactants with excellent wetting ability [96, 97]. Triton™ X series of surfactant vary in molecular weight and number of ethylene oxide units in the structure; for example Triton™ X-100 has an 9 to 10 ethylene oxide

unit per molecule with average molecular weight of 625 g mol^{-1}. Therefore, Triton^{TM} X series varies with solubility and surface acting property depending on the chain lengths of surfactant used; Triton^{TM} X-100 which has a longer chain lights has higher solubility in water at room temperature while Triton^{TM} X-45 has borderline oil-water solubility [97].

1.2.3 Co-Surfactant: Ceteareth-20

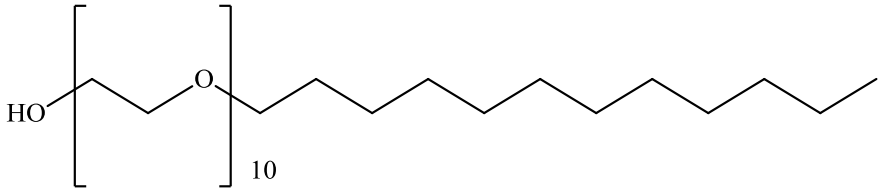


Figure 1.8: Chemical structure of ceteareth-20.

Ceteareth-20 is a polyoxyethylene ethers compound composed of a long lipid tail and ether head group used as emulsifier and thickener to increase viscosity of colloidal system. Unsaturated lipid tail can be packed closely increasing entanglement and increases resistance to flow and hence increases viscosity of colloidal system [98, 99]. Addition of thickener improves fragmentation and increases the kinetic stability of colloidal suspensions consequently slowing down the destabilization process such as sedimentation and particle aggregation [99].

1.3 Emulsion

Emulsification is a process of dispersing immiscible fluids (oil and water) into each other by creation of an interface forming droplets of diameter generally greater than 0.1 μm [80, 100]. Emulsions are heterogeneous liquid mixtures comprising of dispersed medium suspended in a dispersed phase or continuous phase. Emulsion has a milky white, opaque appearance resulting from light scattering and diffuse reflection phenomenon at the phase interfaces of emulsions. Light changes in velocity and refracted when travelled from phases having lower refractive index (water-phase) into phases having higher refractive index (oil-phase). Therefore, when the difference between refractive indices is greater, the changes in velocity of light passing through emulsion would also be greater resulting in higher opacity of emulsion [101, 102].

Emulsions are categorized by phase ratio between oil and water, order of addition and type of emulsifier used. For example, emulsions with volume fraction of oil, Φ_{oil} 0.3 and smaller is an o/w type emulsion, whereas emulsions with volume fraction of oil, Φ_{oil} 0.7 and larger is a w/o type of emulsion. Generally, emulsions are sorted to oil-in-water (o/w), water-in-oil (w/o), water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) emulsions type [103]. Formation of emulsions type are greatly affected by the nature of surfactant and oil type used, mechanical of formation and technique used in blending emulsion [100].

Emulsions do not form spontaneously due to the high interfacial tension and positive free energy which causes them to be thermodynamically unstable [101]. Therefore, external energy input through homogenization, mechanical stirring or shaking is needed for formation of emulsion. Additives such as emulsifiers, finely divided solids and polymers are added into the system to promote excellent long-termed

stability of emulsions [100-103]. Emulsifier adsorbs at the oil and water interfaces creating an energy barrier toward destabilization of emulsions [104].

Destabilization process causes separation of emulsions into respective immiscible phases through mechanisms such as coalescence, flocculation, creaming and Ostwald ripening [104]. Coalescence is an irreversible process taking place when two droplets in contact overcome the energy barrier between them to merge forming larger droplets [104, 105]. Meanwhile, flocculation took place when many droplets combine to form flocci. Flocculation of emulsion droplet near the surface is known as creaming process and sedimentation took place when flocci appear to be at the bottom of emulsions [105]. Ostwald ripening is a process involving diffusion flow of surfactant, droplet with smaller size would dissolve and decreases in sizes reassembling to form larger size droplet (Figure 1.9) [103-105]. Industrial manufacturer governs on variables such as temperature, composition and droplet size distribution to control production of highly stabilized monodispersed narrow size emulsion minimizing possible destabilization processes [104].

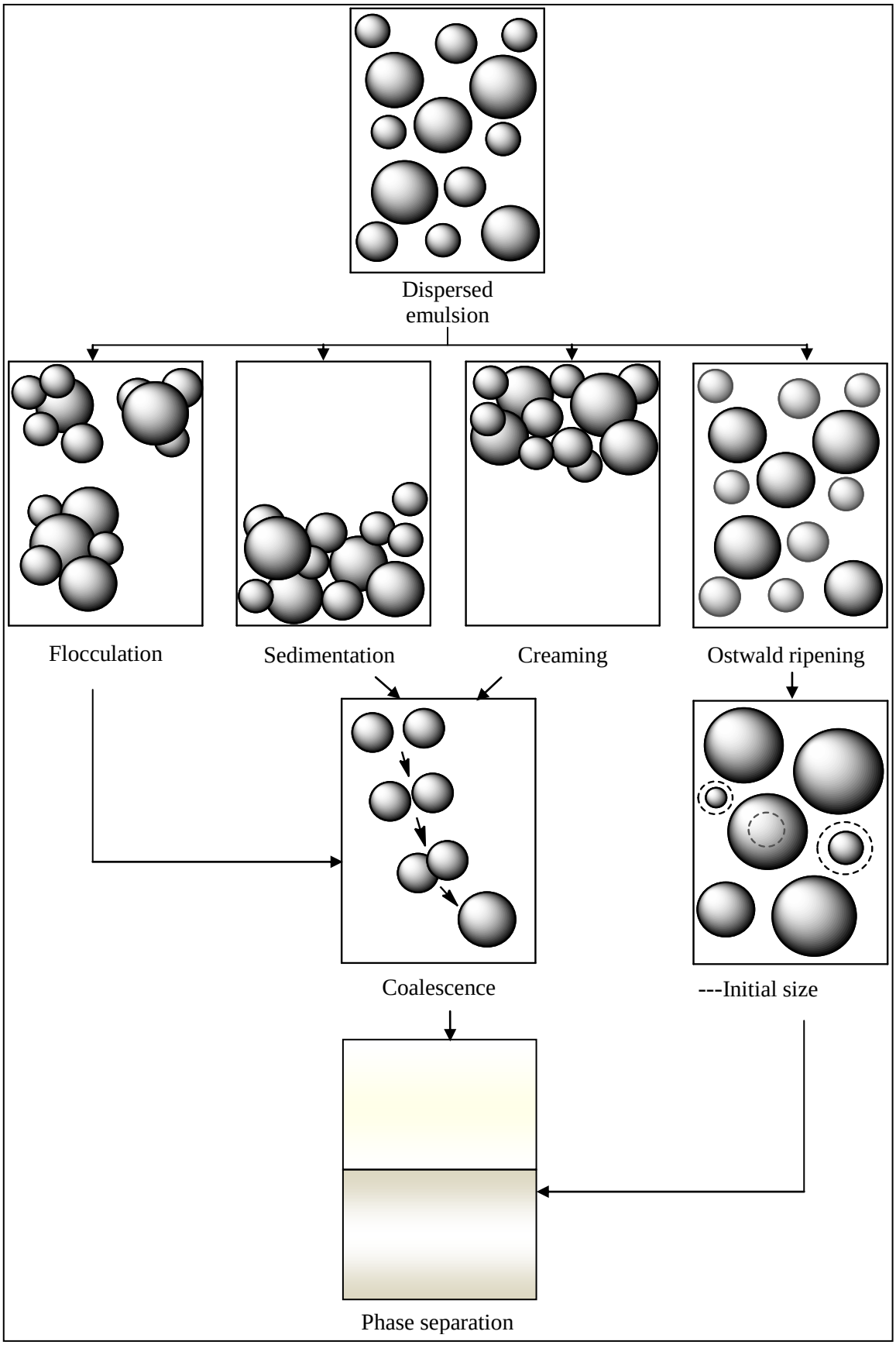
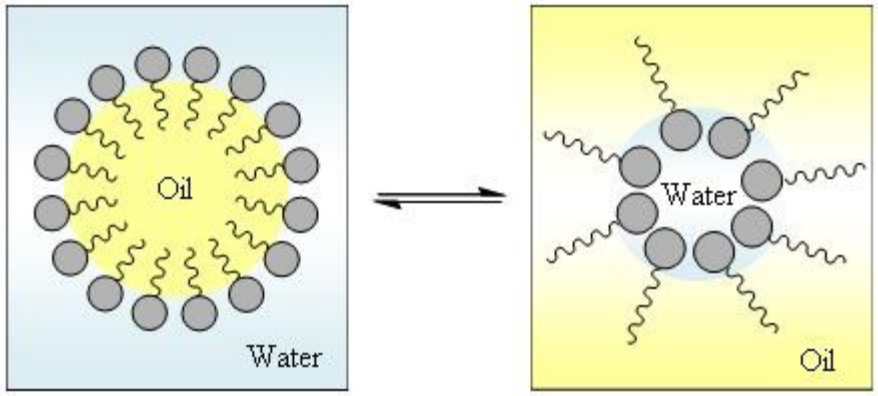


Figure 1.9: Illustration of destabilization mechanism of emulsions adapted from reference [106].

1.3.1 Phase Inversion

Phase inversion occurs when structure of emulsion formed inverted from initial system enclosing the dispersed phase into internal phase of emulsion droplets (Figure 1.10). This process requires low energy input therefore forming emulsions spontaneously producing usually fine and monodispersed emulsions. Therefore, phase inversion method frequently being utilized to produce emulsion industrially [106, 107]. Phase Inversion Temperature (PIT) method is the most frequently used method correlated with spontaneous curvature of surfactant subjected to temperature quench [107].



(a) (b)

Figure 1.10: Illustration of phase inversion of emulsion structure transition from (a) o/w emulsion to (b) w/o emulsion and vice versa, adapted from reference [108].

Changes in physiochemical parameter such as temperature, pressure, salinity, pH of continuous phase, incorporation of co-surfactants, SAD (Surfactant Affinity Difference) and proportion of oil and water takes effect on the spontaneous curvature of aggregation of surfactant at interface causing phase inversion of emulsion droplets [108-113]. SAD is positive for lipophilic surfactant and negative for hydrophilic surfactant and when the empirical number reaches to zero, the surfactant would has the same affinity to both aqueous and oil phases [110-113].

Formulation-composition map is a qualitative tool to evaluate the phase transition of emulsion depending on the emulsification protocol taking into account of surfactant types and the surrounding environment where surfactant is present (Figure 1.11) [114-118]. Central region and transition zones are greatly affected by surfactant concentration where increase in surfactant concentration enlarges both of these regions while mechanical stirring give the opposite effect. Increase in oil phase viscosity favours catastrophic inversion from A+ to C+ region by shrinking A+ region. Whereas, increase of aqueous phase viscosity decreases the A- region endorsing inversion from A- to B- regions. Emulsion can be inverted crossing the line from A+ to A- where the inversion is transitionally governed by dramatic decrease of interfacial tension involving passage through lamellar liquid crystalline phase [118-124]. Whilst, emulsions undergoing catastrophic phase inversion (crossing bold line), progressed through formation of temporary multiple emulsions [114, 125-128].

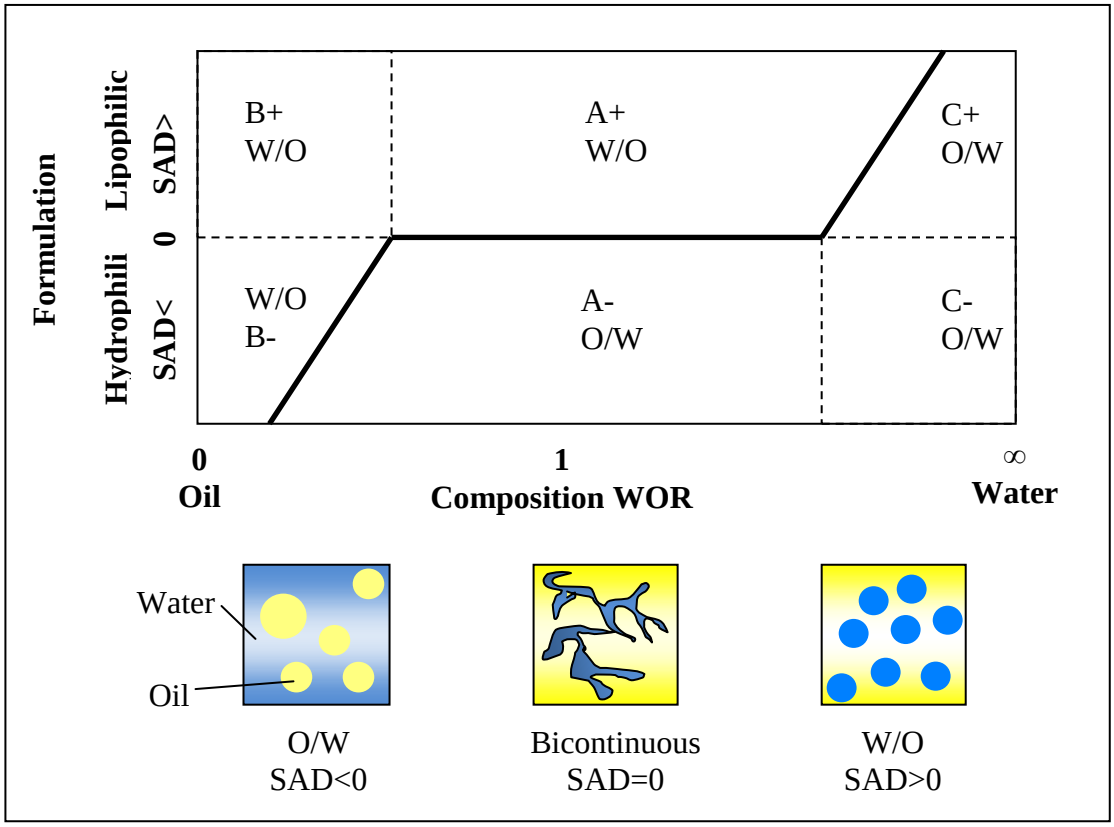


Figure 1.11: Illustration of proposed mechanism for transitional inversion and formulation-composition map adapted from reference [109-113].

1.4 Viscosity

Viscosity is a measurement of resistance of flow of material moving through surfaces. Fluids resist relative motion of layers with differing velocities within them opposing changes in the initial formation generating internal resistance to flow [129]. A fluid showing high resistance opposing flow is said to be thick or viscous whereas fluid having small resistance to flow known as thin or low in viscosity.

Bulk fluid is pictured to consist of a number of thin layers sliding each other with area *A*, separated by fluid distance height *H*, pressed by a force *F*, resulting in the movement of plate with velocity, *V* (Figure 1.12). Newtonian fluid flow is independent on the shear rate which means the shear stress is proportional to shear strain rate (*V/H*), with proportionality constant of dynamic viscosity [130]. Shear stress (τ) is defined as the amount of force applied to sample per unit area, $\tau = \frac{F}{A}$. Shear rate γ is defined as velocity per unit height, $\gamma = \frac{V}{H}$; Viscosity (η) is defined by:

$$\eta = \frac{\tau}{\dot{\gamma}} \tag{1}$$

The viscosity (η) of fluid, is the ratio of shear stress (τ) required to be applied to move the solution at a resulting strain rate ($\dot{\gamma}$) [130].

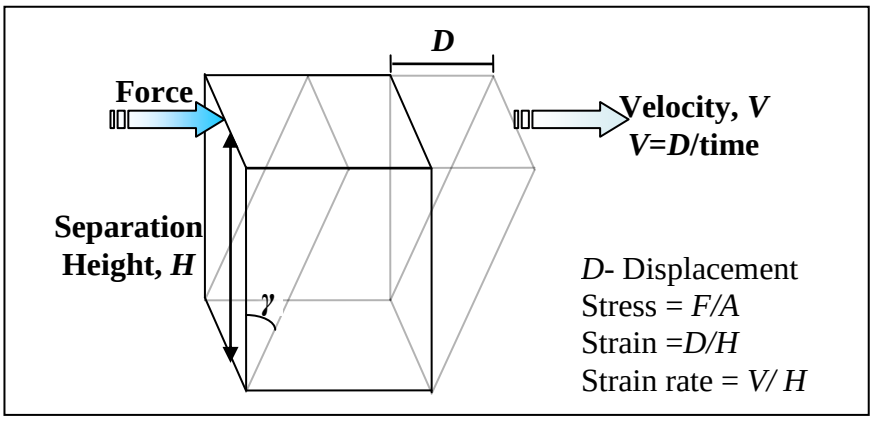


Figure 1.12: Illustration of viscosity quantification picturing a piece of liquid moving at shear rate (γ) under applied shear stress (τ) adapted from reference [130].

1.5 Zeta Potential

Existence of electrical charges comprising of cations (positive charge) and anions (negative charge) in liquid frequently causes unequal distribution of electrical charges across interface creating potential gradient known as electrical double layer. Electrical double layer model is used as a tool to visualize the ionic environment of a charged colloid and the electrical repulsive force exerted on that system. When a positively charged particle is suspended in a liquid, ions with opposite charges (counter-ions) would be attracted to the surface of the charged particle and vice versa [82].

Ions closest to the surface of particle forms a layer of strongly bound inner region known as Stern layer, further from the core lay layers of loosely bound ions forming an outer region known as diffuse layer (Figure 1.13). Within the diffuse layer, there is a notional boundary where particle mobility is related to the dielectric constant and viscosity of suspending liquid to the electrical potential at the boundary (slip plane) between moving particle and the liquid [130, 131].

The unequal distribution causes accumulation and formation of net charge at a particular region balanced by an opposite net charge accumulated at another side maintaining neutrality of electrical charges in the suspension [131, 132]. Consequently, electrokinetic potential is generated giving rise to surface potential with voltage difference in order of millivolts. Magnitude of surface potential decreases rapidly in the Stern layer and reduces exponentially through diffuse layer and approaches zero at imaginary boundary of the double layer. The potential at the slipping plane is called zeta potential [132].

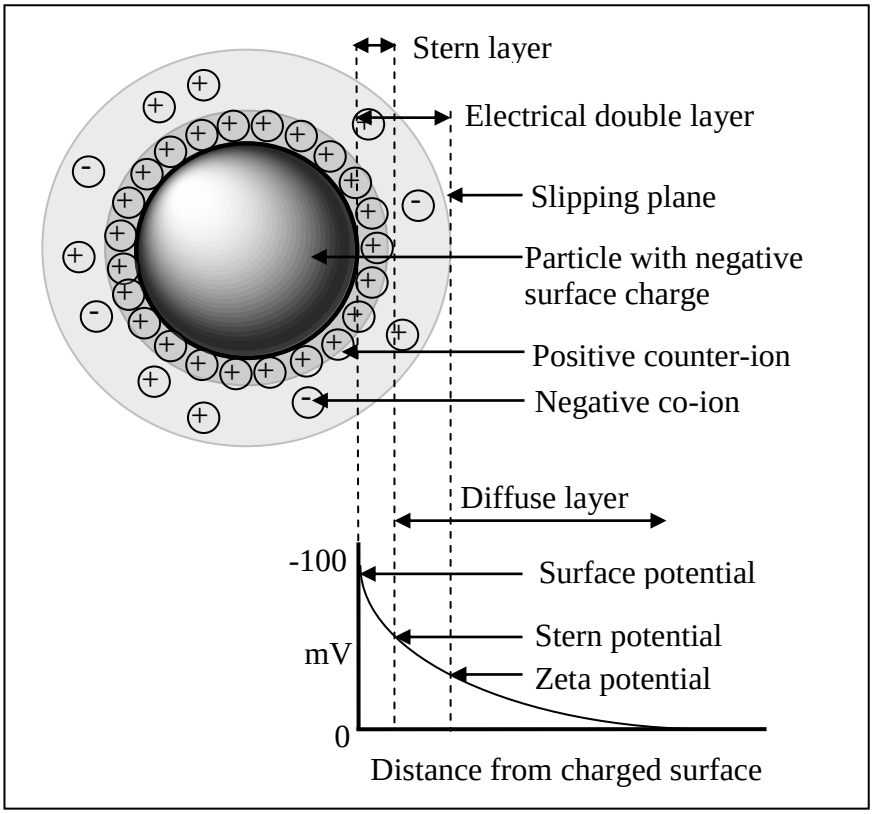
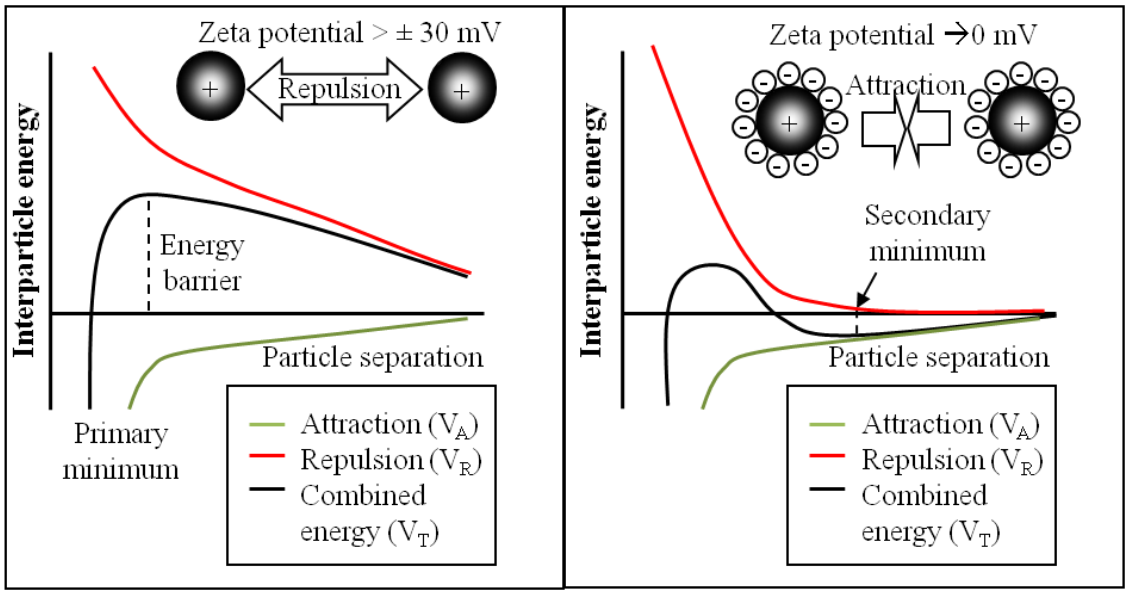


Figure 1.13: Illustration of stern model electrical double layer; distribution of counter-ions in the vicinity of charged surface and variation of electrical potential with increasing distance from charged surface adapted from reference [131].



(a) (b)

Figure 1.14: Illustration representing particle behaviour when exerted with (a) high zeta potential and (b) low zeta potential; variation of free energy with particle separation in suspension adapted from reference [133].

Zeta potential magnitude indicates stability and dispersion of the colloidal system, where particle having large magnitude of zeta potential either large negative or large positive would have a higher tendency to repel each other from flocculate as compared to the lower magnitude of zeta potential (Figure 1.14). Generally, suspensions achieve stability when they achieve zeta potential magnitude of more than ±30 mV [133].

Electrophorestic mobility is obtained through measuring velocity of particle moving at a fixed velocity in a voltage field by using Laser Doppler Velocimetry (LDV) techniques [133]. Using parameters obtained and by knowing the viscosity and dielectric constant of the sample, zeta potential can be determined by applying Henry equation:

$$U_E = \frac{2\varepsilon \zeta f(\kappa a)}{3\eta} \quad (2)$$

Where ζ is zeta potential, U_E is the electrophoretic mobility, ε is dielectric constant, η viscosity and Henry's function $f(\kappa a)$ which is 1.0 or 1.5 [131-133].

Meanwhile, Smoluchowski approximation is used to calculate zeta potential of suspension having particle larger than 0.2 microns dispersed in moderate electrolyte concentration (10^{-3} molar salt) and $f(\kappa a)$ of 1.5 [131-133].

Smoluchowski equation:

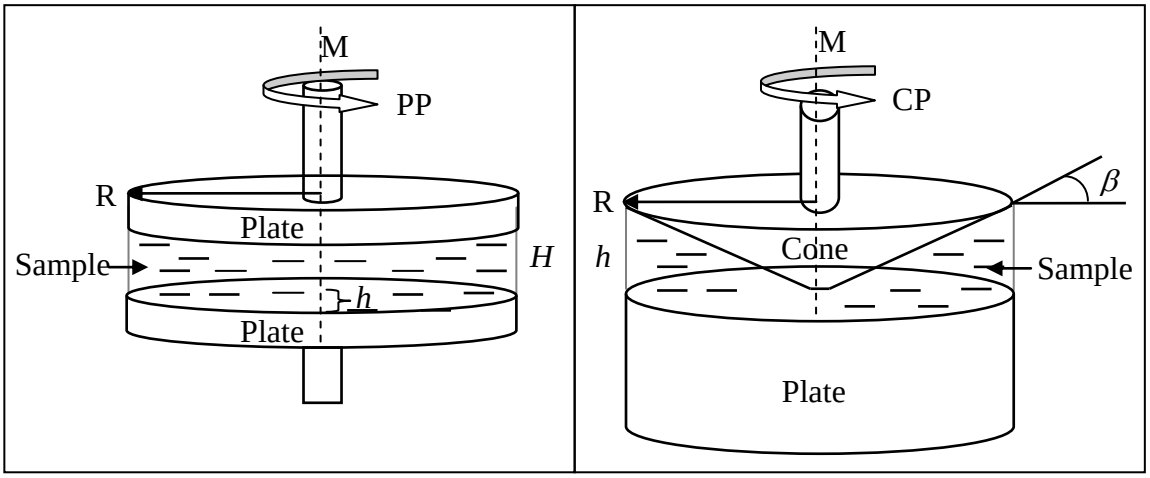
$$\mu = \frac{\zeta \varepsilon}{\eta} \quad (3)$$

Where ζ is zeta potential, μ is the electrophoretic mobility, ε is electric permittivity and η is the viscosity of liquid [133].

1.6 Rheology

The study of material reaction toward applied force is known to be rheology. Rheology describes the flow of fluids and deformation of solids under stress and strain [134] plays a significant role in evaluating the ability of material to perform efficiently at a specific function and temperature in industrial applications [132]. Evolution of rheometer leads to the invention of more sophisticated rheometers enabling measurement of forces ranging from less than 1 mNm to more than 1 Nm with temperature range of -150 °C to 300 °C [134].

Rheological measurement is used to characterize cosmetic products into categories of creams, lotions and gels determined from the flow behaviour, yield point, critical shear rate and onset of flow of the product [135]. Emulsion stability is governed mostly by strength of interparticle interactions, hydrodynamic forces, volume fraction, electrostatic forces, steric repulsion, Brownian motion, size and shape of interacting particles in suspensions. By measuring rheology of emulsions, interaction of colloidal substances undergoing destabilization processes such as flocculation, coalescence resulting in undesired settling and separation of emulsions can be predicted [136].



(a) (b)

Figure 1.15: Illustration of (a) parallel plate; (b) cone and plate measuring system adapted from reference [134].

Cone and plate (CP), Figure 1.15 (b) measuring system is favourably used to characterise emulsion system attributed to the sample viscosity, while parallel plate (PP) Figure 1.15 (a) measuring system is applicable to solid and semi-solid system and cylindrical system is used on sample with low viscosity [137]. Cone geometries are modelled as a cone with tip pointing towards the bottom plate with cone angle of β.

$$\tan\beta = \frac{h}{R} \tag{4}$$

R is defined as radius, *h*, a variable plate gap and angular speed in the gap ω (*h*). For small angle of β, tan β can be adjusted to be equal to β resulting constant shear rate at every point on the surface of the cone in CP model [136, 137].

$$\dot{\gamma} = \frac{\omega}{\tan\beta} = \frac{\omega}{\beta} \tag{5}$$

Most materials possessed intermediate characteristic of fluid like (viscous) and solid like (elastic) which is known as viscoelastic material. Solid or fluid material is subjected to deformation with application of an external force. Deformation caused by shear stress is permanent while elastic displacement which is the relative displacement of material without destroying the cohesion of material withholding the ability to return to its original form with removal of force. Force leading to deformation of sample is defined by applied torque [137, 138].

Rheology of viscoelastic material can be described by a simple mechanical model consisting of one dash pot and spring connected in series which is Maxwell model type fluid flow behaviour (Figure 1.16). Ideal Maxwell like viscoelastic fluids are characterized by semicircle shaped Cole-Cole plot represented by storage modulus or elastic modulus, *G'* and loss modulus or viscous modulus, *G"* [138, 139]. Phase angle, tan δ is equivalent to $\tan^{-1}$ (*G'/G"*) and ranges from 0° to 90º (Figure 1.17). The lower the phase angle value the more solid like is the material and vice versa. For phase angle below 4º, *G'* response is dominating *G"* response at all measured frequency, this

suggest that the elastic solid like behaviour dominates over the liquid like viscous property. The coupling of low phase angle and linear increase in *G'* indicates higher degree of emulsion and longer life storage [139].

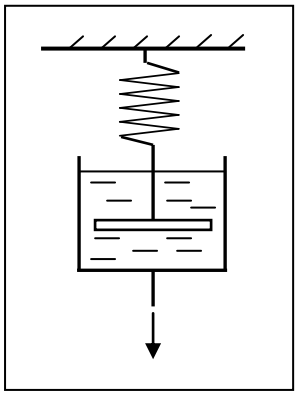


Figure 1.16: Maxwell model [10].

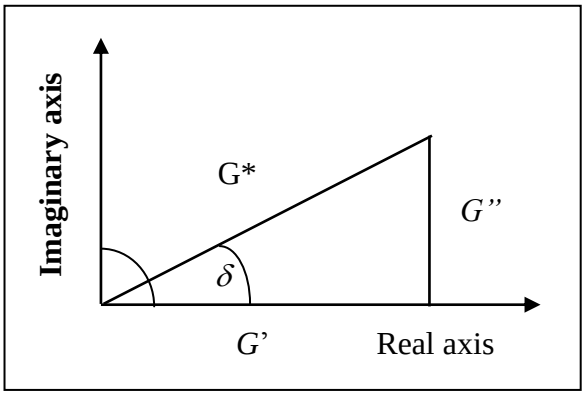


Figure 1.17: Plot of complex modulus G* [10].

Geometries rotating continuously in one direction deformed materials in a steady measurement mode which run at a controlled shear stress or at variable shear stress. Velocity and preset shear stress or strain obtained from the measurement is used to calculate the dynamic viscosity. Dynamic measurement or oscillation measurement is the application of oscillating shear stress or shear strain on sample in a rotational sinusoidal system [140].

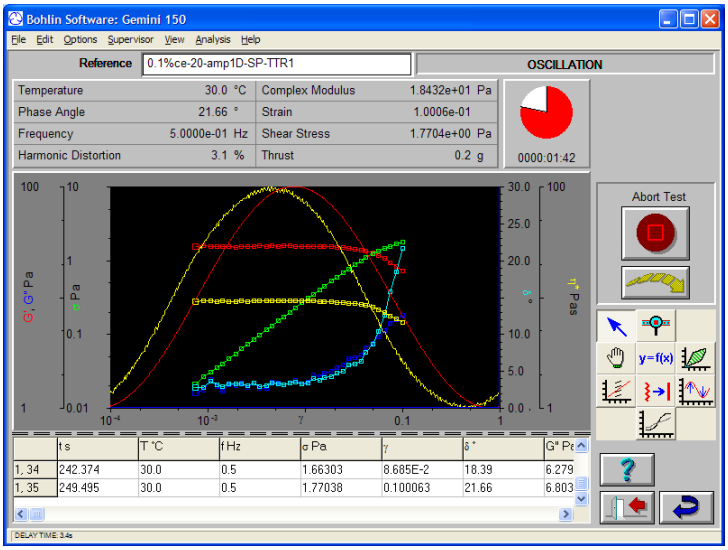


Figure 1.18: Diagram showing real time oscillation measurement of amplitude variation performed at constant temperature and frequency (*f*).

Amplitude test or strain test would usually be subjected to initial sample testing essential in determining linear viscoelastic region (LVR) where *G'* modulus and *G"* modulus are nearly parallel in the lower frequency range (Figure 1.18). Strain test is conducted in small step increasing amplitude. *G'* and *G"* would give a constant response with increasing amplitude until a point where *G'* would decrease dramatically giving value lesser than *G"* indicating sample started to flow [141, 142].

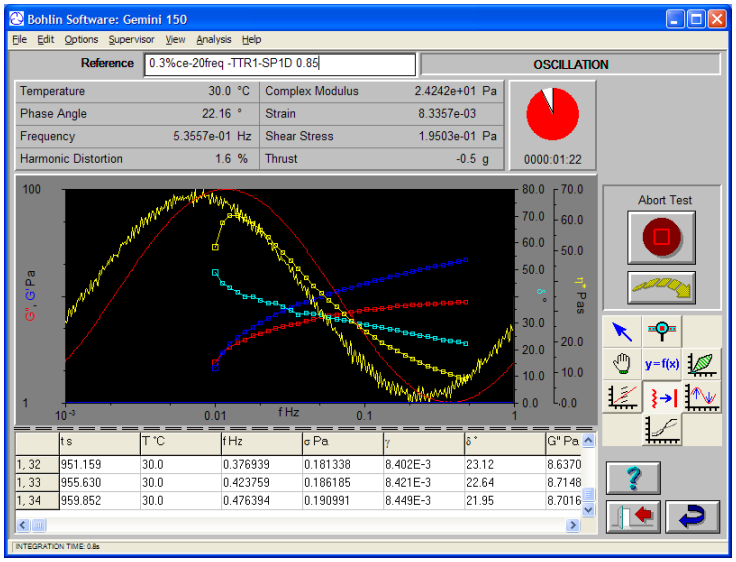


Figure 1.19: Diagram showing real time frequency measurement with frequency variation performed at constant temperature and strain ($\dot{\gamma}$).

Figure 1.19 shows an example of frequency test conducted with increasing frequency at a fixed temperature and strain determined from LVR. Frequency test was performed after determining the LVR of sample, to ensure that the two measurements are comparable and with the same boundary condition, as amplitude at the non-linear region would yield different results in analysis producing unreliable results. In addition, a rheometer has limitation when measured near the torque resolution with error of 100%, while the minimum required accuracy is expected to be less than 10%, therefore, the frequency range must be limited appropriately to produce reliable results [140-142].

1.7 Objective of Studies

- To study the stability of essential oil emulsion stabilized by glycolipid surfactants;
- To study the particle size and zeta potential of essential oil emulsion;
- To investigate the physical properties of essential oil emulsion through rheological (elastic, viscoelastic and viscous) properties and microscopic analysis.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

Cosmetic grade essential oils, white Thyme oil (W306509), Tea tree oil (W390208), Lavender oil 40/42% fluers (W262218) and Citronella oil (Java, natural, 85/35%; W230812) purchased from Sigma-Aldrich Sdn. Bhd. were used in emulsion formulation. Barnstead Diamond Nanopure Water Purification unit connected to Barnstead Diamond^{TM} RO unit from Barnstead International, Iowa USA producing deionized water with resistivity of 18.2 Ω cm^{-1} was used in water phase preparation of emulsion system. Food grade Laleli extra virgin olive oil distributed by Thye Huat Chan Sdn. Bhd. was used as oil base in emulsion formulation. Glycolipid surfactants categorized as sucrose stearate, sucrose palmitate and sucrose myristate were obtained from Mitsubishi-Kagaku Food Corporation. Triton^{TM} X-100 and Triton^{TM} X-45 used to stabilise emulsion system were obtained from Sigma-Aldrich Sdn. Bhd. Ceteareth-20 supplied by KC Chemical (M) Sdn. Bhd. was used to increase the viscosity of emulsion system. Sodium Chloride, NaCl used in zeta potential measurement is obtained from R&M Chemicals.

2.2 Experimental Technique and Preparation

2.2.1 Emulsion preparation

Aqueous phase was prepared by solubilising 1.00 to 10.0 wt% of glycolipid surfactants (SS, SP, SM) and 0.01 to 0.10 wt% of ceteareth-20 into 50 wt% of deionized water per total volume. Vortex mixing and ultrasonication were applied on mixtures for 2 minutes respectively followed by heating at 60 °C in a water bath until a colourless clear gel phase was obtained. Oil phase was prepared by mixing solution of 12.5 wt% of

essential oil, 0.001 to 0.010 wt% Triton^{TM} X-100 and Triton^{TM} X-45 per total volume of emulsion; ratio of Triton^{TM} X-100 to Triton^{TM} X-45 used varies according to the type of essential oil used. Oil phase was transferred into aqueous phase and homogenized at 13,000 rpm for 10 minutes with SilentCrusherM Homogenizer Heidolph Instrument, Germany. Emulsion formed as then transferred and kept in an incubator of 45.0±0.1 °C subjected to accelerating ageing process. Stability of emulsion is determined from observing emulsion's physical changes for a period of 30 days involving instability and separation of emulsion into oil and aqueous phases.

2.3 Instrumentation

2.3.1 Viscometer

LAUDA Processor viscosity measuring system PVS connected over a personal computer as a central control unit managing viscosity measuring autonomous functional unit was used to measure viscosity of essential oil, carrier oil and mixture of essential oil. Automated kinematic viscosities determination was carried out using standardised capillary viscometers with optimum adaptation to the requirement of application with respect to sample. All measurements were carried out at a regulated temperature of 30.0±0.1 °C.

2.3.2 GC-MS

Gas chromatography (GC) coupled with mass spectrometry (MS) instruments (GC-MS, model QP-2010 Shimadzu, Japan) was used in analysis of compositions of essential oils. Thyme and tea tree were diluted 1:100 (v/v) with methanol, while lavender oil and citronella were diluted 1:100 (v/v) with hexane. 1.0 μL of diluted samples was automatically injected in splitless mode to SGE BPX 5 capillary column (30.0 m, 0.25 mm i.d., 0.25 μm film thickness with carrier gas of helium at a flow rate of 1 mL min^{-1}). Injector and detector temperatures were set at 250 °C and 280 °C respectively. Column temperature was set to 60 °C for 5 minutes, then gradually increased to 160 °C at 4 °C min^{-1} and finally increased to 270 °C at 15 °C min^{-1}. GC peaks were identified by comparing fragmentation pattern of MS with those reported in the literature and reference compound available [143].

2.3.3 Polarizing Microscope

Light polarizing microscope from Leica model PM RXP by Leica Microsystems GmbH, Germany was used to observe the emulsion droplets formed. Polarizing microscope unit was equipped with a high voltage beam, polarizing unit and a JVC Color Video camera with model KY F550, interfaced with personal computer with Leica QWin image analysis software. Leica QWin software is a powerful tool used in digital images capturing with capability of measuring and analysis of droplet size of sample. Observation of changes in droplet size and appearance of emulsion has been made for 30 days.

2.3.4 Zetasizer

Emulsion samples of 0.001 wt% are dissolved in 25 ml of 0.0001 mol dm^{-3} NaCl, the solutions was equilibrated at a constant temperature of 30.0±0.1 °C in a water bath. The sample was then filled up into a cuvette and inserted into the Malvern Zetasizer Nano Series (Malvern Instruments, UK). Zeta potential measurement was carried out consecutively on the 1^{st}, 3^{rd}, 7^{th}, 14^{th} and 30^{th} days of emulsion prepared. Smoluchowski approximation was applied in the calculation of zeta potential as emulsion was dispersed in moderate electrolytes concentration of more than 10^{-3} molar salt and droplet size greater than 0.2 microns and $f(\kappa a)$ is 1.5 [131-133].

2.3.5 Rheometer

Rheological evaluation was carried out on essential oil emulsion prepared for 1^{st}, 3^{rd}, 7^{th}, 14^{th} and 30^{th} days by using Bohlin CVO-R Rheometer Germini^{TM}, Malvern Instrument UK with temperature regulator of Peltier Plate system (-40 °C to 180 °C, from Bohlin Instrument Ltd.) [10]. Rheometer system consists of a triple-mode control which allows strain, stress or shear rate controlled measurement to be performed by using a single test station. A built-in normal force sensor is used for ranges of measurement, gap control and sample loading protocols enabling fine measurement of deformations, speeds and viscoelastic responses of material. Bohlin CVO-R Rheometer Germini^{TM} was optimized for strain controlled and stress controlled operation with extensive range of temperature control options broadening the applications in product development [10, 131, 133]. All measurements were conducted at a constant temperature of 30.0±0.1 °C, using cone and plate (CP 4/40, cone diameter of 40 mm, 4° angle and gap width of 0.150 mm) geometry. Steady dynamic viscosity tests were

carried out at a controlled increasing shear rate, γ ranging from 0.0001 to 70 s^{-1} to determine viscosity η, of samples, while the corresponding shear stress τ were measured. Amplitude sweep was performed prior to frequency sweep to ensure both test were in the same boundary condition ensuring reliability of data. Amplitude sweep was performed at a controlled strain mode ranging from 0.0009 to 0.8 units with a frequency of 0.5 Hz. As for frequency sweep, storage modulus *G'* and loss modulus, *G"* were measured as a function of frequency varied from 0.001 to 10 Hz.

CHAPTER 3: RESULTS AND DISCUSSIONS

3.1 Viscosities and Densities of Oil

Viscosity measurements were carried out on several types of olive oil and essential oils by using automated LAUDA PVS measuring system at temperature of 30.0±0.1 °C. Viscosity of liquid is a measurement of friction of liquid molecules sliding past each other, which gives the numerical information of fluids resistance to flow [144, 145]. Dynamic viscosity (absolute viscosity) is a ratio of applied shear stress to resulting strain rate, implementing the amount of shear stress applied to move liquid at a particular strain rate expressed in centipoise (cP) or S.I. unit mPa s. Kinematic viscosity was determined by assessing the flow time of a known volume of fluid flowing through calibrated capillary tube (Ubbelohde viscometer) at controlled temperature [129]. Kinematic viscosity is the product of measured flow time and calibration constant of the viscometer expressed in centistokes (cSt.) or S.I. unit $mm^2\ s^{-1}$ [145]. The kinematic and dynamic viscosities are related as follows:

$$\nu = \frac{\eta}{\rho} \tag{6}$$

where, ν is the kinematic viscosity, η is the dynamic viscosity, and ρ is the density [144, 145].

Table 3.1: Measurements of viscosity and density of olive.

No.	**Sample (Olive oil)**	**Kinematic Viscosity 10^2 (±0.005 $mm^2\ s^{-1}$)**	**Density (±0.001 $g\ cm^{-3}$)**	**S.G (±0.001)**	**Dynamic Viscosity 10 (±0.005 mPa s)**
1.	Olive oil, Fluka (75357)	0.597	0.906	0.910	0.541
2.	Olive oil, Highly refined Sigma-Aldrich, (O1514)	0.599	0.908	0.912	0.544
3.	Extra virgin olive oil, Laleli	0.559	0.906	0.910	0.507

Table 3.2: Measurements of viscosity and density of essential oil.

No.	Sample (Essential oil)	Kinematic Viscosity 10^2 (±0.005 $mm^2 s^{-1}$)	Density (±0.001 $g\ cm^{-3}$)	S.G (±0.001)	Dynamic Viscosity 10 (±0.005 mPa s)
1.	Thyme oil, White, FCC, (W306509)	0.036	0.916	0.920	0.033
2.	Lavender oil 40/42% fleurs, (W262218)	0.033	0.883	0.887	0.029
3.	Tea tree oil, (W390208)	0.026	0.888	0.892	0.023
4.	Citronella oil, Java, natural, 85/35%, (W230812)	0.057	0.880	0.884	0.050

Table 3.3: Measurements of viscosity and density of essential oil and olive oil mixture.

No.	Sample	Kinematic Viscosity 10^2 (±0.005 $mm^2 s^{-1}$)	Density (±0.001 $g\ cm^{-3}$)	S.G (±0.001)	Dynamic Viscosity 10 (±0.005 mPa s)
1.	Olive oil : Thyme oil 75% : 25%, (3 : 1)	0.295	0.909	0.913	0.269
2.	Olive oil : Lavender oil 75% : 25%, (3 :1)	0.249	0.899	0.903	0.224
3.	Olive oil : Tea tree oil 75% : 25%, (3 : 1)	0.228	0.902	0.906	0.206
4.	Olive oil : Citronella oil 75% : 25%, (3 : 1)	0.271	0.898	0.903	0.244

Table 3.1 shows the measurement of viscosity, η of the carrier oil (olive oil) with different brand, grades and country of origin. Refined olive oil from Sigma-Aldrich shows the highest viscosity followed by Fluka, olive oil and Laleli, extra virgin olive oil. Viscosity of oil is closely correlated with the degree of unsaturation and chain length of fatty acids in triglycerides. η increases with the degree of polymerisation and decreasing degree of unsaturation [145]. The linear organisation of saturated

hydrocarbon enables the fatty acid chains to be lined up closely having van der Waals inter-particle interactions and reducing the flowability resulting in high oil η. Meanwhile, unsaturated fatty acid having a double bond arranged in cis configuration forming a 'bent' structure, preventing packing of lipid chain overall reducing the inter-particle contacts consequently increases the capability to flow with decreasing η [146].

Extra virgin olive oil has the lowest η, suggesting a lower degree of polymerisation and presence of higher number of unsaturated fatty acids. Refined oil was chemically treated and refined from virgin olive oil, therefore possesses higher number of saturated fatty acid chain causing higher η. Olive oil having intermediate η was a blend of virgin olive oil and refined oil [147]. Although higher viscosity oil possessed greater affinity in emulsion formation, but due to safety reason, nutritional value and environmental issue, food grade extra virgin olive oil categorized as the highest quality of olive oil was selected as the carrier oil base for this project [11, 12].

Essential oil varies in viscosity and density depending on the chemical component such as short chain phenolic compound, terpenes, terpenoids esters, ethers and alcohols which are present in the extract (Table 3.2) [147, 148]. Viscosity of the essential oils is relatively lower than the viscosity of the olive oil by 10 fold, hence, by mixing the oil component, the overall η of mixture increases and causes emulsion formations to be more viable (Table 3.2 and Table 3.3) [148].

By referring to Table 3.3, thyme oil mixtures (0.269±0.005)10 mPa s displays the highest dynamic viscosity value followed by citronella oil mixture (0.244±0.005)10 mPa s, lavender oil mixture (0.224±0.005)10 mPa s and tea tree oil mixture (0.206±0.005)10 mPa s. Since phenol group of molecule is a stronger organic acid than ketones, esters, ethers and etc., formation of hydrogen bond is more viable and readily available [148]. Thyme oil consists mainly of phenolic monoterpenes [149], therefore they possess the highest interaction with fatty acid from carrier oil through

formation of hydrogen bonding which eventually increases the resistivity of flow and η of the mixtures.

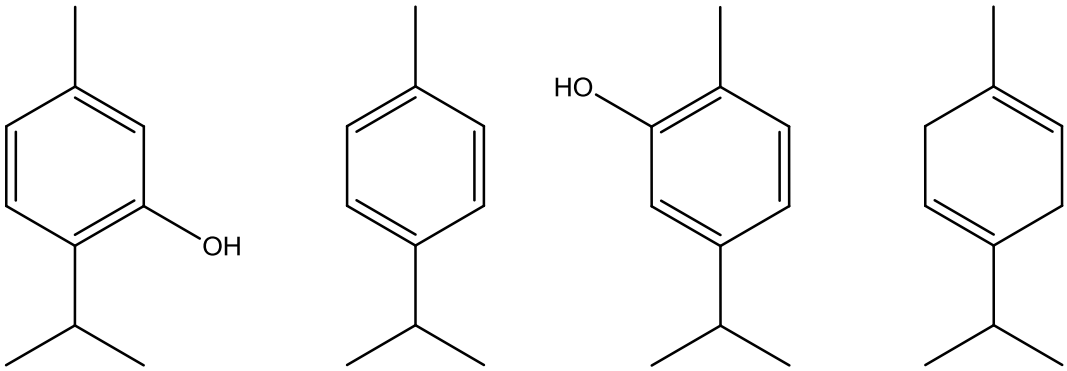
Citronella oil consists mostly of short chain hydrocarbon compounds comprises of monoterpenes, hydrocarbons, ketones, aldehyde and esters (neral and geranial) [19], suggesting lower resistance compared to thyme oil due to lower entanglement of compound mixture. Citronella oil contains more oxygen groups and unsaturated hydrocarbon than tea tree oil which is made up of more than 70% of terpineol-4-ol, γ-terpinene and α-terpinene [55]. Oxygen groups and unsaturated double bond promotes formation of hydrogen bonding between carrier oil and essential oil [148]. Therefore, it possesses higher dynamic viscosity than those of tea tree oil and lavender oil.

3.2 Essential Oil Emulsion System

Using 100% of essential oil in oil phase of emulsion formulation causes emulsion droplet formed to be inverted as shown in Figure 3.2 (b). Initially, lipid tail of glycolipid surfactants were being arranged in a manner where they surround an oil core forming emulsion system with oil droplet suspended in aqueous phase (o/w) as in illustration Figure 3.2 (a). Short chain, bulky hydrocarbon molecule from essential oils (eg. *p*-cymene, β-caryophyllene, thymol, and terpinen-4-ol) shows incompatibility with long chain glycolipid surfactant, as the molecules could not accommodate the long lipid tail of surfactant causing high steric repulsion between lipid tails in emulsion droplet. This, forces lipid tail to emerge out to the surrounding reservoir in order to reduce steric forces resulting in spontaneous rearrangement of glycolipid surfactants, inverting emulsion droplet forming w/o emulsion when external force was being removed from the system.

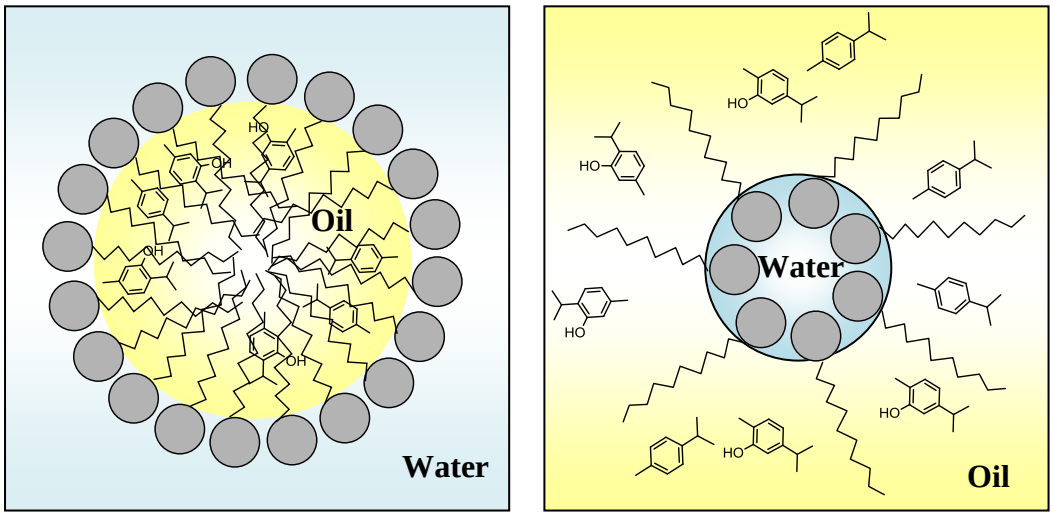
Table 3.4: Compositions of essential oils (%) from thyme, lavender, tea tree and citronella by using GC-MS.

Component	Composition (%)			
	Thyme	**Lavender**	**Tea Tree**	**Citronella**
Hydrocarbons				
α-pinene	1.64	0.93	3.33	-
α-terpinene	0.16	-	3.88	3.71
p-Cymene	25.91	-	1.35	-
γ-Terpinene	9.41	-	5.27	0.72
β-caryophyllene	-	5.45	3.63	6.37
β-cis ocimene	-	5.82	-	-
Terpinolene	-	-	1.09	-
Limonene	-	-	0.64	7.98
Germancrene-Δ	-	-	-	1.14
Alcohols				
Linalool	9.41	31.37	-	2.56
Borneol	2.16	2.69	-	1.33
Terpinen-4-ol	2.24	6.15	59.74	-
α-terpineol	2.53	2.53	8.95	0.72
β-Citronellol	-	-	-	16.68
Nerol	-	-	-	21.43
ethers and ketones				
1,8-cineole	-	5.15	2.32	-
Camphor	0.90	-	-	
Geraniol	-	4.04	-	0.88
(R)-(+)- Citronellal	-	-	-	26.08
Phenols				
Thymol	38.53	-	-	-
Carvacrol	1.20	-	-	-
Esters				
Linalyl acetate	-	24.56	-	-
Lavandulol acetate	-	2.96	-	-
Geranyl acetate	-	-	-	5.13
Neryl acetate	-	-	-	1.44
Others	5.91	8.35	9.8	4.13
Total	**100**	**100**	**100**	**100**



Thymol *p*-Cymene Carvacrol γ-Terpinene

Figure 3.1: Molecular structures of major chemical compounds present in thyme oil [19, 150, 151].



(a) (b)

Figure 3.2: Illustrations of emulsion droplet formation, (a) initial o/w emulsion system and (b) inversion of emulsion droplet, w/o emulsion system.

To overcome this problem, a carrier oil which acts as a medium having long lipid tail compensating the short chain molecule of essential oils was being introduced into the emulsion system. Carrier oil acts as diluents of essential oils, diluting the concentration of essential oil and increasing the viscosity of oil mixture and promoting emulsion formation. Food grade Laleli selection extra virgin cold pressed olive oil has been selected as the carrier oil for this emulsion system. Extra virgin olive oil was selected due to the presence of monounsaturated and polyunsaturated fatty acid [152]

with major component constituted of oleic acid [153]. Presence of long chain fatty acids aids the formation of essential oil emulsion by creating medium in allocating glycolipid surfactants which overall reduces the steric repulsion between lipid tails. Hence, stabilizing the emulsion droplet and preventing inversion of emulsion droplets.

Addition of co-surfactant such as Triton^{TM} X-100 and Triton^{TM} X-45 possesses structure compatibility with phenolic group which is present in essential oil giving rise to greater interaction between the molecules. Having a shorter chain length, Triton^{TM} X-45 has a higher compatibility with shorter chain terpenes present in essential oil. Apart from that, ether tails of Triton^{TM} X-100 envelopes the oil droplet in oil phase which increases the solubility of the oil phase in water further enhancing the formation of essential oil emulsion [97].

However, due to the short side chain of essential oil molecule, emulsion formed appeared to be low in viscosity even though supporting material such as carrier oil, surfactant and co-surfactant have been added into the system. Hence, ceteareth-20 is selected as an additive to increase the viscosity of the emulsion formed. Long lipid tail of ceteareth-20 increases viscosity of emulsion by creating resistance and lowering the chances of emulsion droplet from sliding through each other [98]. Increased viscosity induces kinetic stability of suspension by slowing down the rate of sedimentation and particle aggregation. Presence of ether group provides structural compatibility and formation of hydrogen bonding between the oil group increases viscosity of the emulsion [98, 99]. Addition of ceteareth-20 into the emulsion system stabilizes the emulsion system formed up to 30 days of storage.

3.3 Dispersed Phase of Emulsion System

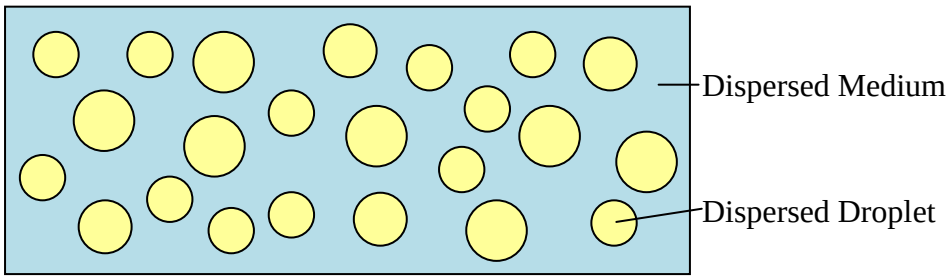
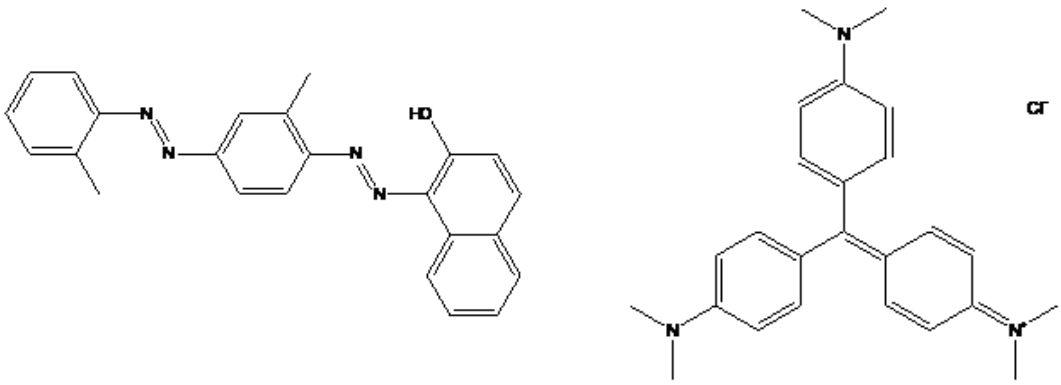


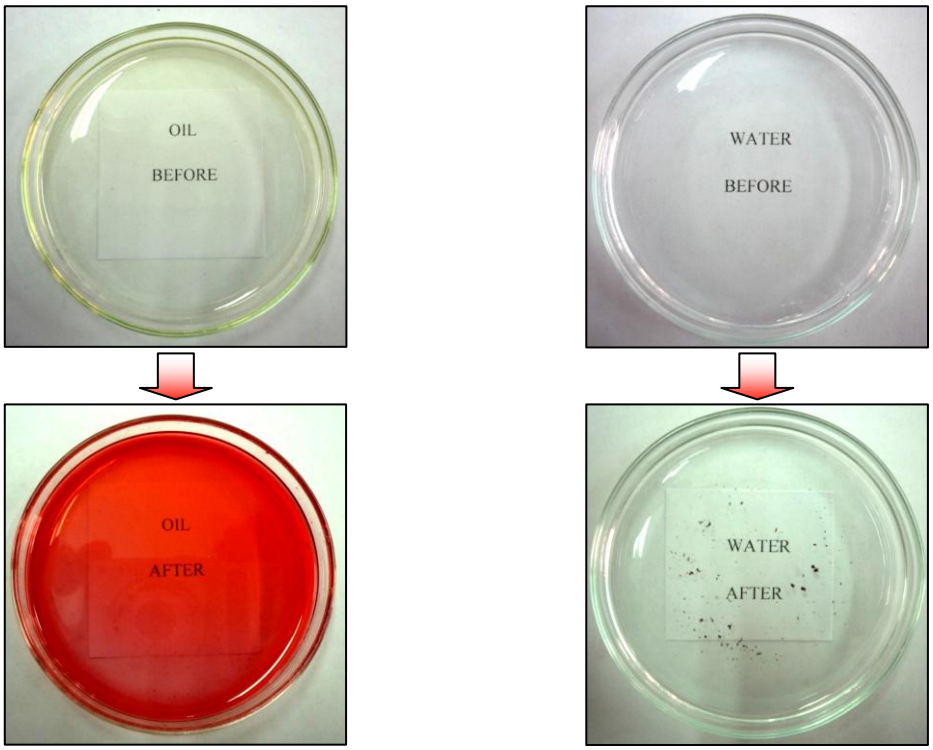
Figure 3.3: Illustration of emulsions droplets dispersed in continuous phase.

Emulsion system dispersed phase was determined by using dye solubility test [154]. This test was conducted to determine the dispersing medium or continuous phase of the emulsion and identification of emulsion system which is either o/w or w/o system. Two types of dyes were selected in this experiments, the dye selected was of opposite nature; soluble only in aqueous phase or oil phase. Sudan IV, an oil soluble dye and crystal violet, water soluble dye were selected in the test [155-157].



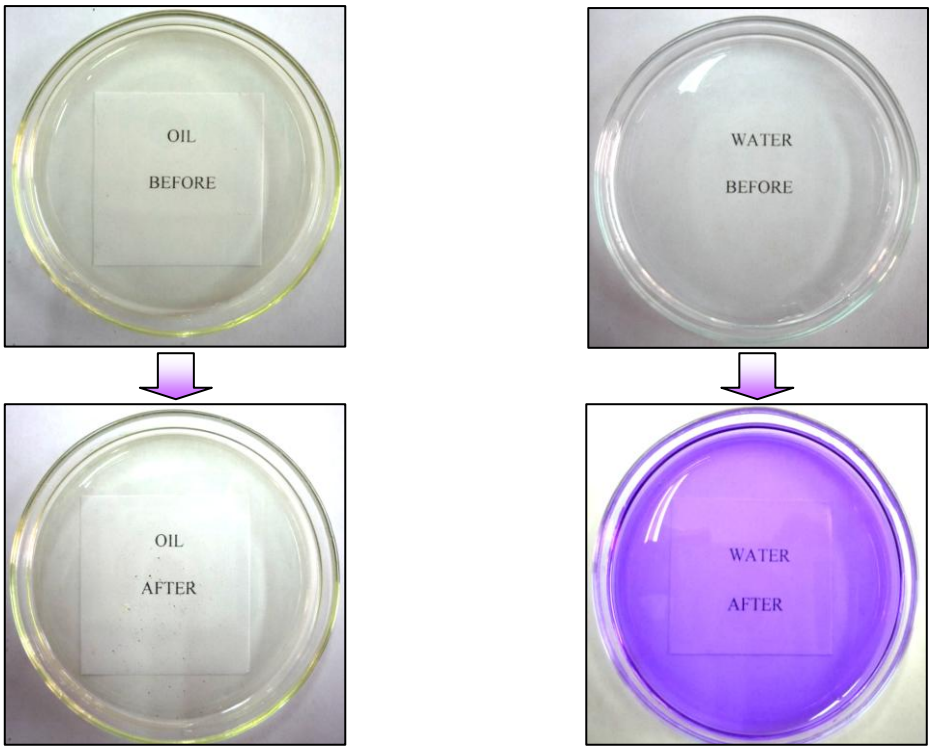
(a) (b)

Figure 3.4: Molecular structure of (a) Sudan IV and (b) crystal violet [156, 157].



(a) (b)

Figure 3.5: Solubility of Sudan IV in (a) oil medium and, (b) aqueous medium.



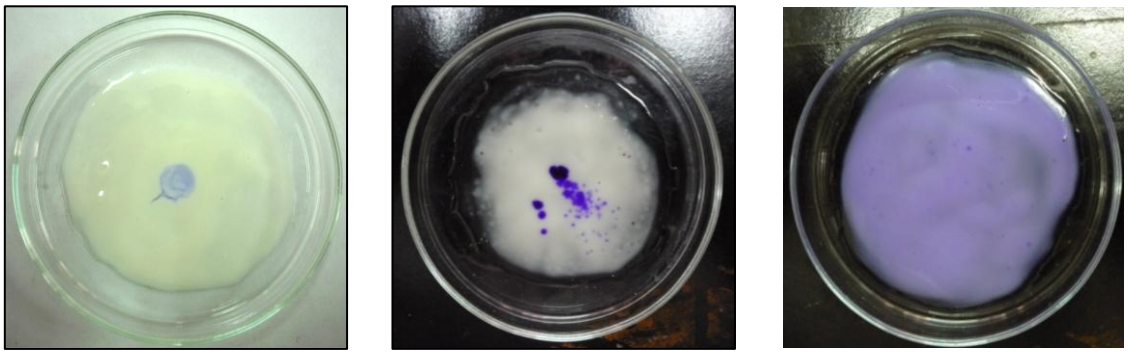
(a) (b)

Figure 3.6: Solubility test of crystal violet in (a) oil medium and, (b) aqueous medium.

Figure 3.5 proves that Sudan IV was an oil soluble dye as they dissolve in oil medium to give a red solution. Figure 3.6 shows the diagram of crystal violet observed to dissolve only in water to give violet solutions. Sudan IV and crystal violet remain as solid particles undissolved in water and oil medium respectively. Continuous phase of emulsion was determined by mixing dye into the emulsion, the solubilised dye in dispersed medium would give information on the nature of emulsion system, either o/w or w/o emulsion system. Sudan IV and crystal violet were added into the emulsion system in three different ways; (a) dissolved dye added into emulsion, (b) addition of solid dye without stirring and (c) addition of solid dye with stirring.

(a) (b) (c)

Figure 3.7: Diagram showing Sudan IV in emulsion with addition of (a) solubilised Sudan IV in oil (b) solid Sudan IV without stirring and (c) solid Sudan IV with stirring.



(a) (b) (c)

Figure 3.8: Diagram showing crystal violet in emulsion with addition of (a) solubilised crystal violet in water, (b) solid crystal violet without stirring and (c) solid crystal violet with stirring.

Results show that the drop of Sudan IV dye does not show sign of spreading, Figure 3.7 (a) suggesting that the dispersed phase of the emulsion is not an oil base. Further testing is carried out on the emulsion by addition of Sudan IV solid directly to the emulsion without stirring Figure 3.7 (b), and with stirring Figure 3.7 (c). Both of the tests show no sign of spreading and solubility of the dye. Hence, the dispersed medium of the emulsion system is not an oil base system.

Figure 3.8 (a), show the spreading of crystal violet solutions in emulsion implying that the dye can be dispersed into the continuous phase suggesting that the dispersing medium was water. Figure 3.8 (b) shows solid crystal violet dyes being added directly without stirring, Figure 3.8 (c) with the aid of stirring. Crystal violet was observed to dispersed, distribute and dissolves in the continuous phase of the emulsion which further validate water to be the dispersed medium of emulsion, concluding essential oil emulsion is an o/w emulsion system.

3.4 Stability Test

A series of stability test had been carried out on essential oil in emulsion by varying parameters such as concentration of glycolipid surfactants, oil to water ratio and concentration of co-surfactants (Triton^{TM} X-45, Triton^{TM} X-100 and ceteareth-20). Essential oil emulsion has been subjected to accelerated ageing process at 45.0±0.1 ºC and observation on destabilization of emulsion by phase separation has been carried out for the duration of 30 days, greater phase separation suggests that emulsion system is prone to destabilization process, having lower integrity and shorter shelf-life.

3.4.1 Stability Test of Thyme Essential Oil Emulsion

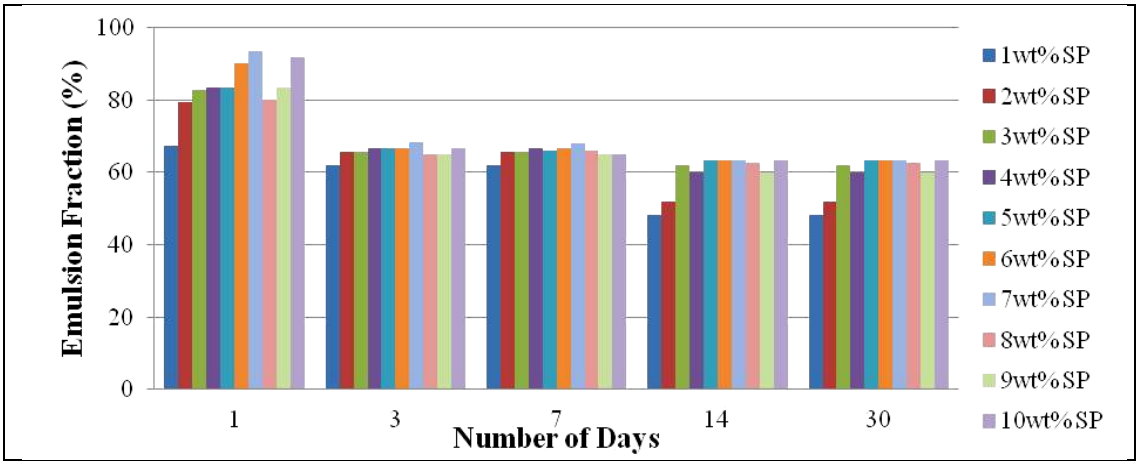


Figure 3.9: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% thyme oil, o:w = 1:1; variation of 1-10 wt% sucrose palmitate glycolipid surfactants.

Figure 3.9 is showing phase separation of thyme essential oil emulsion with varying surfactant concentration. All emulsions show nearly equal phase separation except for 1 wt% and 2 wt% SP surfactant emulsions. Emulsion formations was greatly affected by the amount of surfactant blended into the system, insufficient amount of surfactant used would cause ineffectiveness of emulsion formation. While large amount of surfactant added would cause wastage and saturation of surfactant. Therefore, a system with 5 wt% of glycolipid surfactant was selected as optimum for further formulation.

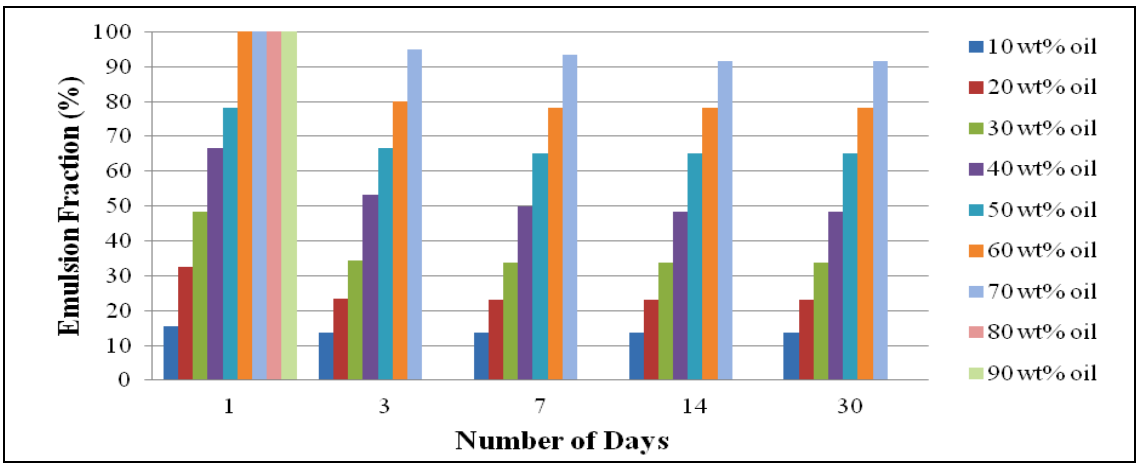


Figure 3.10: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% thyme oil, 5 wt% SP; variation of oil percentage in total volume of emulsions from 10% to 90%.

Ease of formation of emulsion increases with increased oil fraction in the emulsion system until o:w = 8:2 and o:w = 9:1 (Figure 3.10). Emulsion formed from 80 wt% and 90 wt% of oil was not sustainable as emulsion would be reverted to oil and aqueous phase giving a full phase separation. Therefore, emulsion system which have a balance of oil to water ratio of 1:1 with 50 wt% oil in total volume of emulsion was chosen as optimum o:w ratio for essential oil emulsion system. Emulsion with o:w ratio of 1:1 forms relatively high emulsion fraction compared to those of lower oil percentage and possessed higher stability over emulsions with high oil fractions.

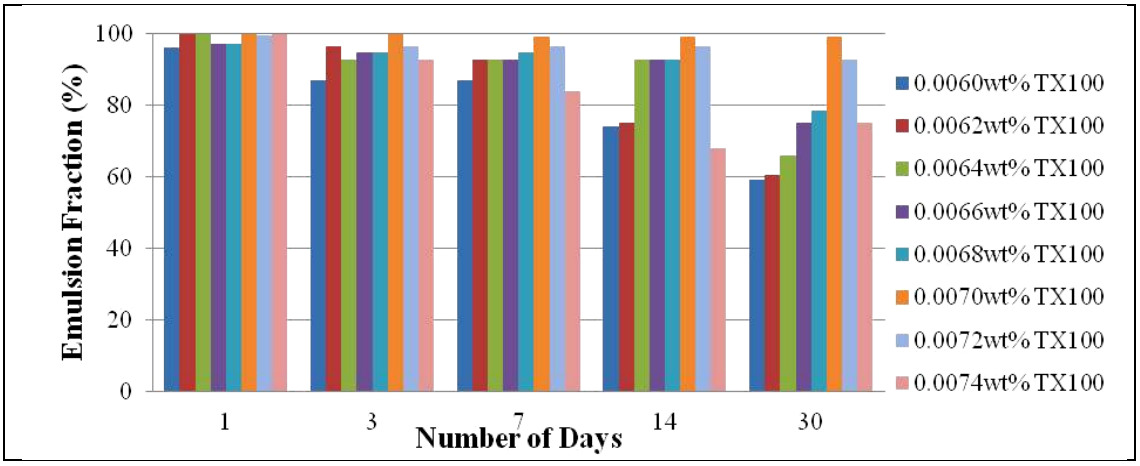
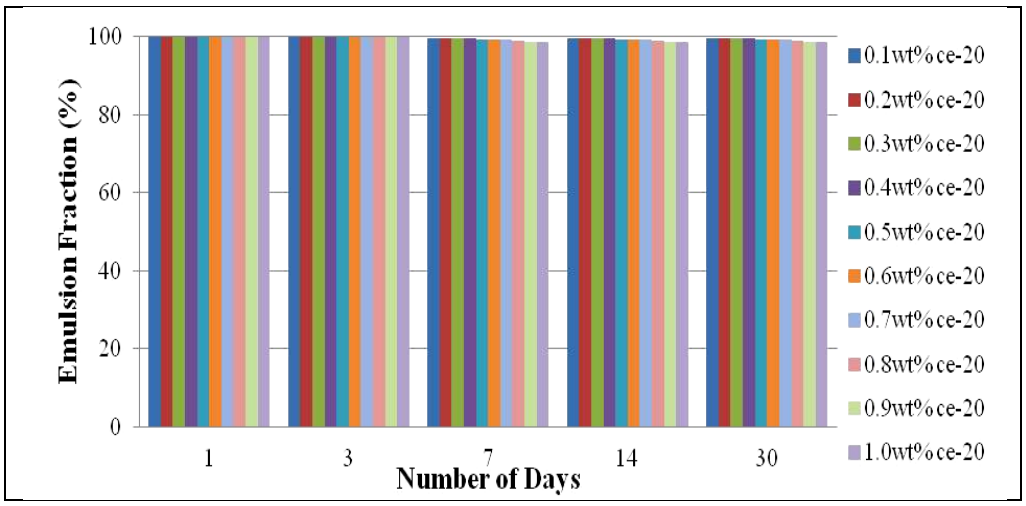


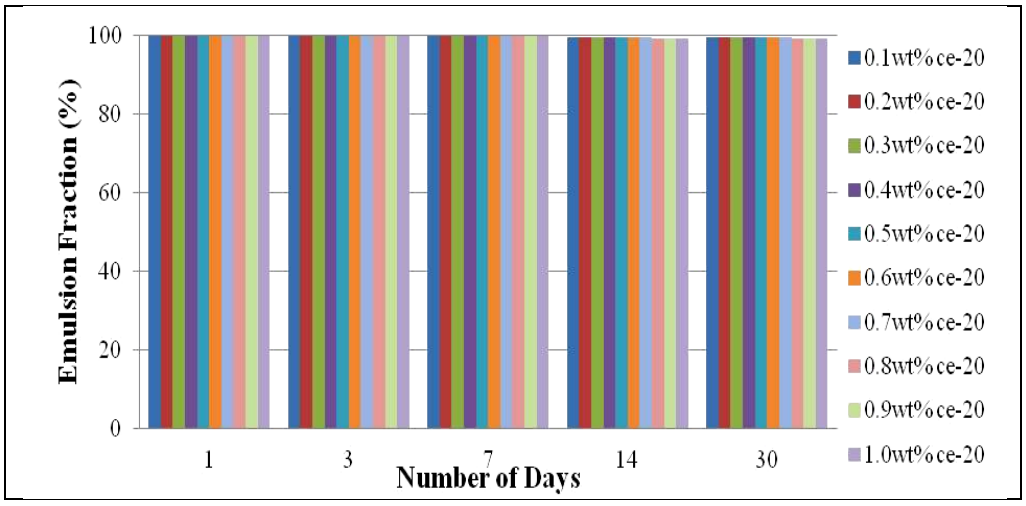
Figure 3.11: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% thyme oil, 5 wt% SP, o:w = 1:1; variation of 0.006-0.0074 wt% Triton^{TM} X-100.

Triton^{TM} X-100 which is structurally compatible with essential oil, emulsion containing 0.007 wt% of Triton^{TM} X-100 presents the highest emulsion fraction which was sustainable up to 30 days of storage (Figure 3.11). Figure 3.12 shows stability of emulsion formed with 0.1-0.5 wt% of ceteareth-20. With the aid of ceteareth-20, stability has been greatly enhanced with increased in continuous phase viscosity which results in kinetic stability of emulsion [158]. Formula of thyme essential oil was determined to be 5 wt% of glycolipid surfactant, 12.5 wt% of thyme oil in total volume of emulsion, o:w ratio of 1:1, 0.007 wt% of Triton^{TM} X-100 and 0.2 wt% of ceteareth-20.

(a)



(b)



(c)

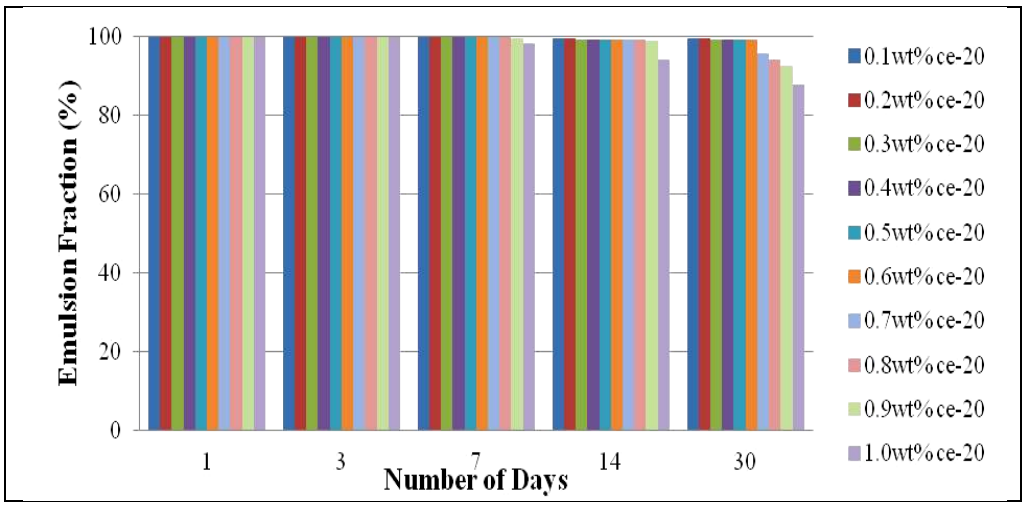


Figure 3.12: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% thyme oil, o:w = 1:1, 0.007 wt% Triton^{TM} X-100; variation of 0.1-1.0 wt% Ceteareth-20 for 5 wt% (a) SS, (b) SP, (c) SM.

3.4.2 Stability Test of Lavender Essential Oil Emulsion

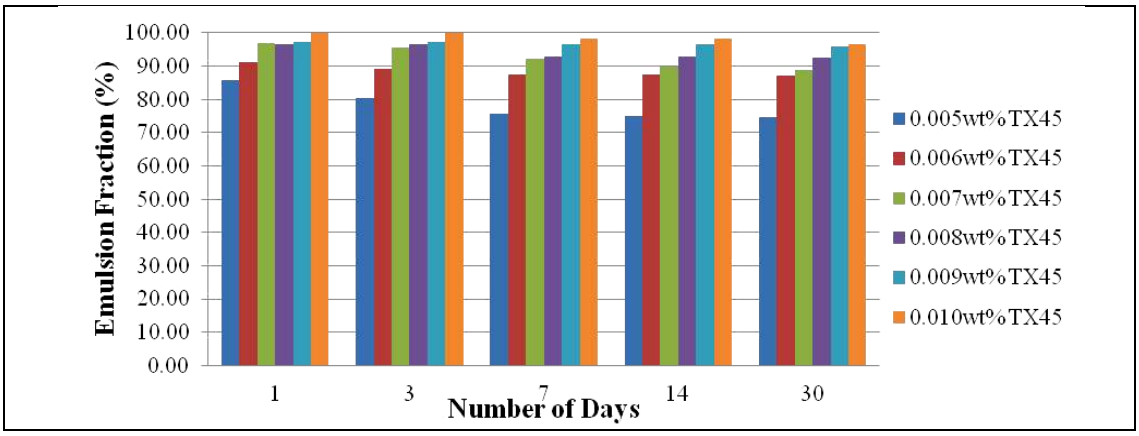
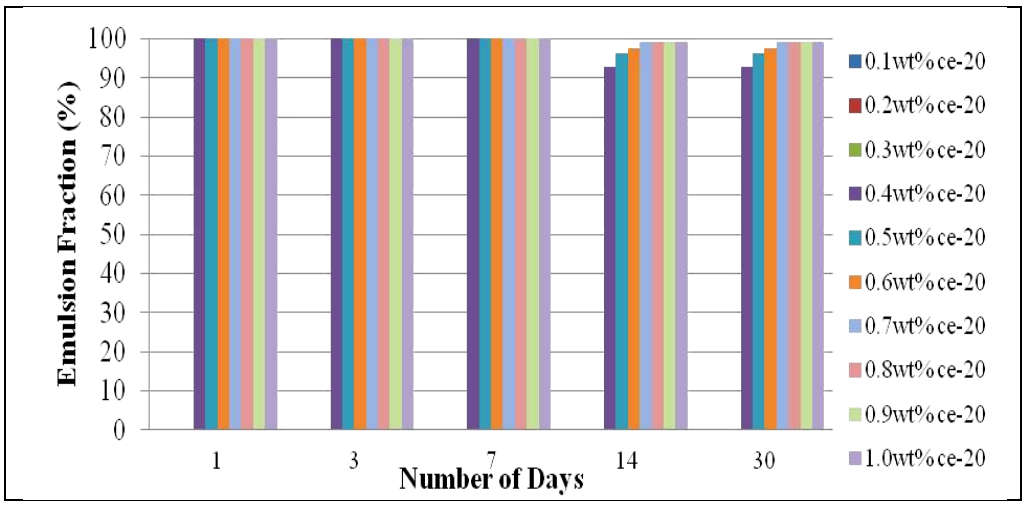


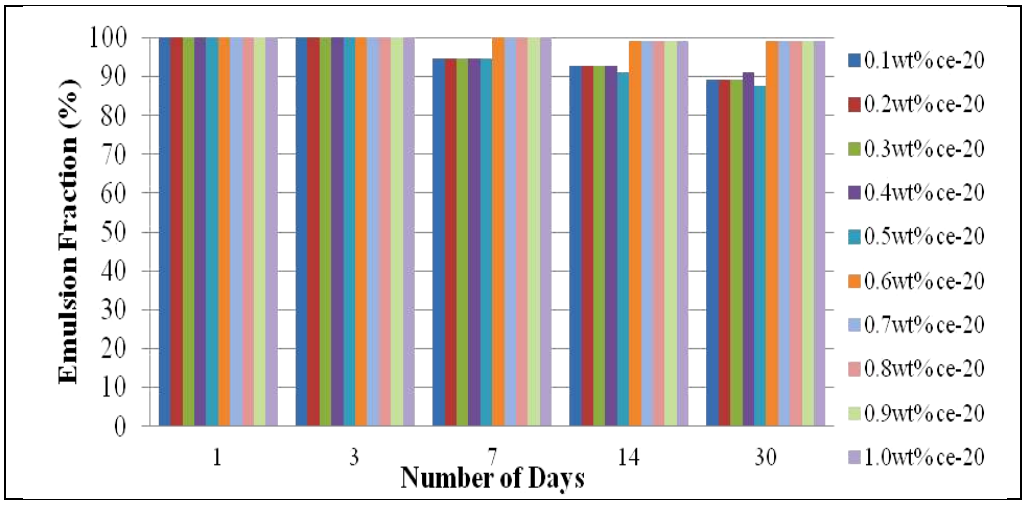
Figure 3.13: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% lavender oil, 5 wt% SP, o:w = 1:1, 0.002 wt% Triton^{TM} X-100, 0.2 wt% ceteareth-20; variation of 0.005-0.010 wt% Triton^{TM} X-45.

Subsequent essential oil formulation was regulated with respect to the thyme emulsion formula, retaining parameters such as o:w ratio (1:1) and the amount of glycolipid surfactants (5 wt%). Lavender oil, comprises mostly of terpenes and terpenoids [34, 35] and they form weaker interactions with fatty acids present in carrier oil as formation of hydrogen bond was not viable as compared to phenolic compound found in thyme oil. Therefore, Triton^{TM} X-45 having a shorter lipid tail was added to create greater interaction with short chain hydrocarbons of lavender oil, resulting in greater van der Waals interactions, increasing the viscosity of the oil phase, consequently forming stable emulsion. Figure 3.13 shows enhanced emulsion formation with increasing amount of Triton^{TM} X-45 from 0.005-0.010 wt% with 0.010 wt% Triton^{TM} X-45 providing the highest emulsion fractions over 30 days of observation. Figure 3.14 shows 0.1-0.6 wt% of ceteareth-20 in emulsion which shows a slight decrease of emulsion fractions starting from 7th day till 30th day and 0.7-1.0 wt% ceteareth-20 in emulsion show improve viscosity and stability of emulsion. Formulation with 12.5 wt% Lavender oil, o:w = 1:1, 0.010 wt% Triton^{TM} X-45, 0.002 wt% Triton^{TM} X-100, and 0.7 wt% ceteareth-20 shows promising emulsion formation sustainable up to 30 days of storage.

(a)



(b)



(c)

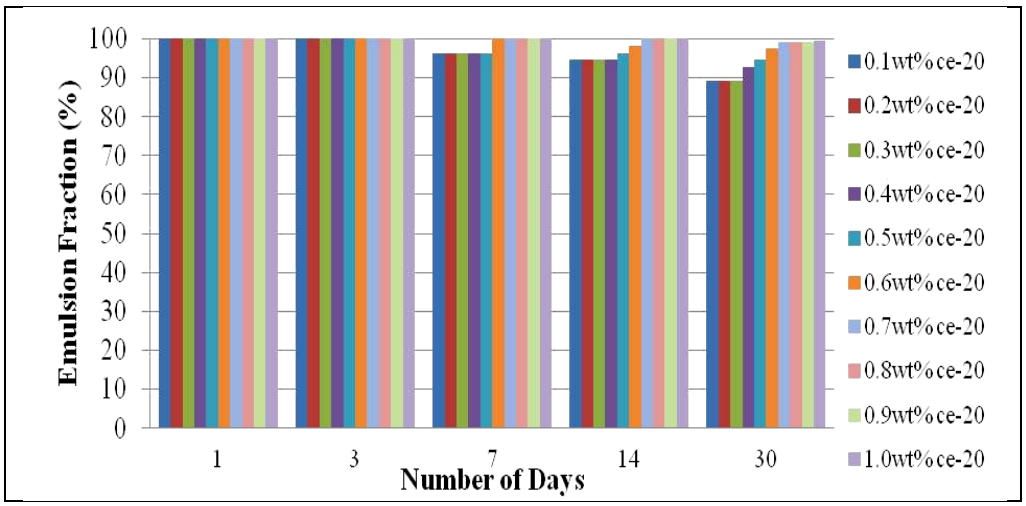


Figure 3.14: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% lavender oil, o:w = 1:1, 0.010 wt% Triton™ X-45, 0.002 wt% Triton™ X-100; variation of 0.1-1.0 wt% ceteareth-20 for 5 wt% (a) SS, (b) SP, (c) SM.

3.4.3 Stability Test of Tea Tree Essential Oil Emulsion

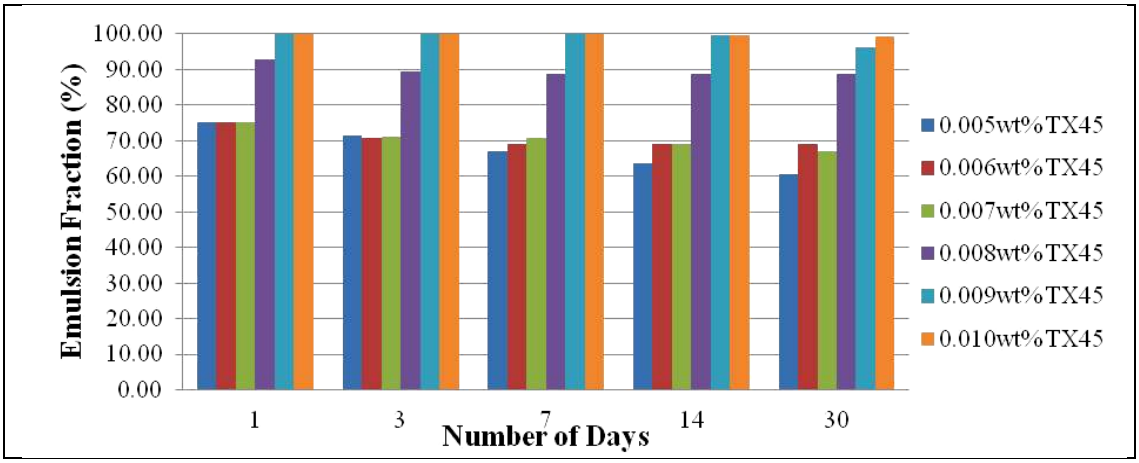
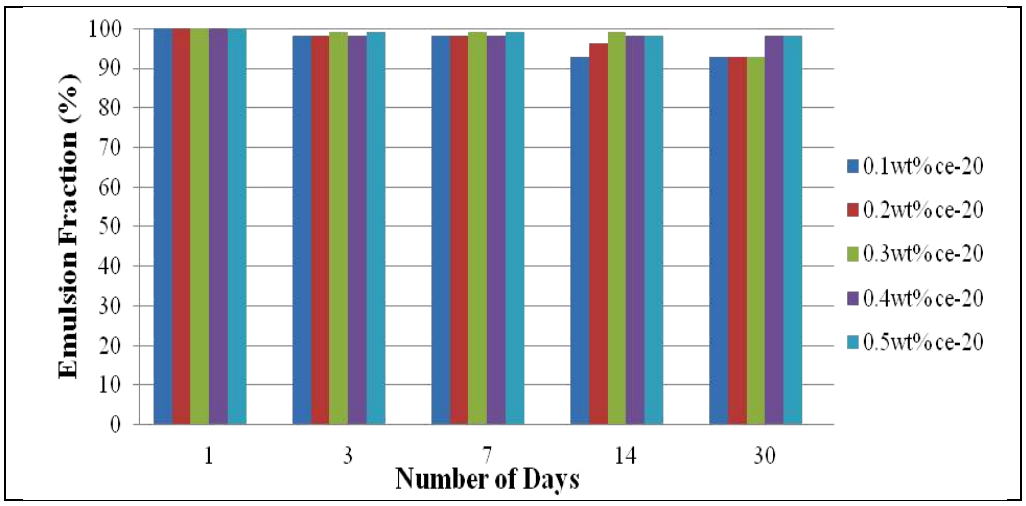


Figure 3.15: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% tea tree oil, 5 wt% SP, o:w = 1:1, 0.004 wt% Triton^{TM} X-100, 0.2 wt% ceteareth-20; variation of 0.005-0.010 wt% Triton^{TM} X-45.

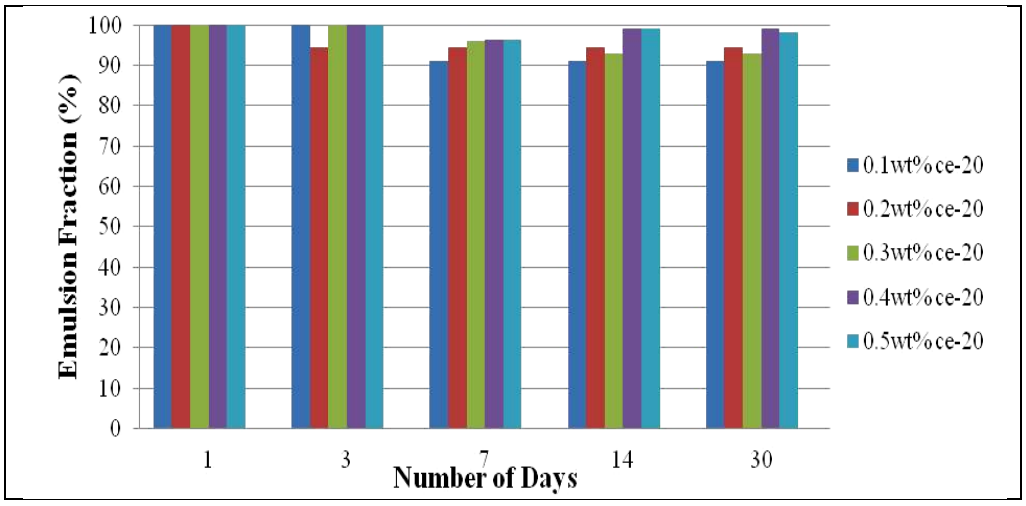
Tea tree oil shows greater interaction with carrier oil as they consist mostly of terpenes and tertiary alcohol [43-45] withholding the ability to form hydrogen bonding displaying higher emulsion fractions with 0.009-0.010 wt% Triton^{TM} X-45 (Figure 3.15) requiring 0.004 wt% of Triton^{TM} X-100 to stabilise the system. Although, 0.010 wt% Triton^{TM} X-45 displayed high emulsion fraction for both SS and SP, but SM was destabilized at 14th day after formation (Figure 3.16). Overall, emulsion formed from 0.010 wt% Triton^{TM} X-45 exhibits lower value of emulsion fraction than that of 0.009 wt% Triton^{TM} X-45.

Emulsion fractions were recorded high for emulsion with 0.009 wt% Triton^{TM} X-45 for all the glycolipid surfactants used with minor destabilization experienced by SM emulsion. SM emulsion shows decreasing emulsion fractions from 0.1-0.5 wt% ceteareth-20 with greatest separation at 0.5 wt% ceteareth-20. Therefore, the most favourable emulsion formula obtained for tea tree emulsion was 12.5 wt% tea tree oil, o:w = 1:1, 0.009 wt% Triton^{TM} X-45, 0.004 wt% Triton^{TM} X-100 and 0.2 ceteareth-20.

(a)



(b)



(c)

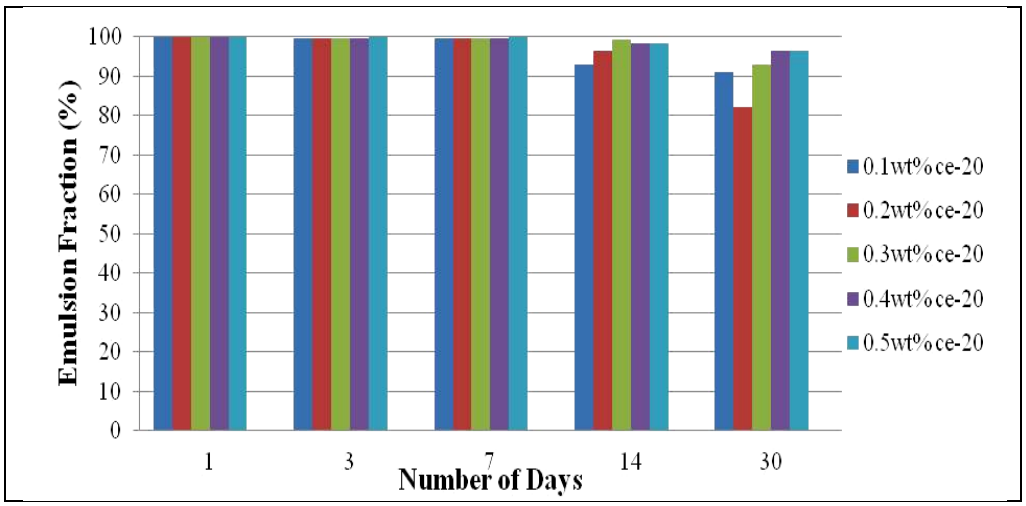
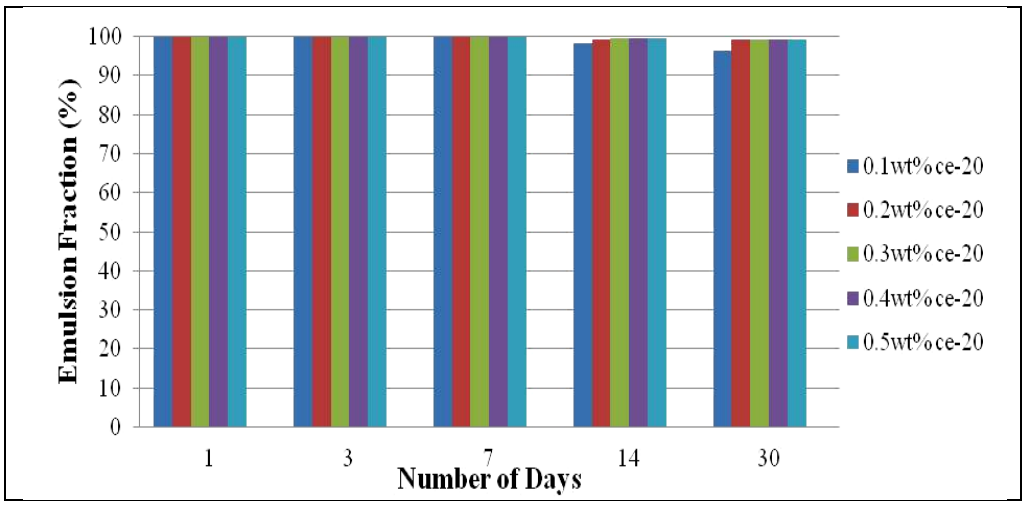
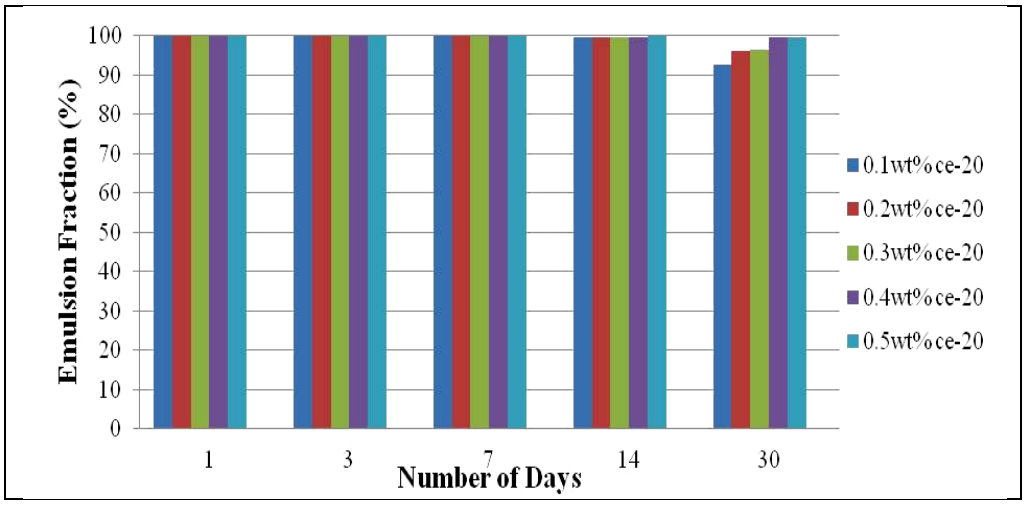


Figure 3.16: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% tea tree oil, o:w = 1:1, 0.010 wt% Triton^{TM} X-45, 0.004 wt% Triton^{TM} X-100; variation of 0.1-0.5 wt% ceteareth-20 for 5 wt% (a) SS (b) SP and (c) SM.

(a)



(b)



(c)

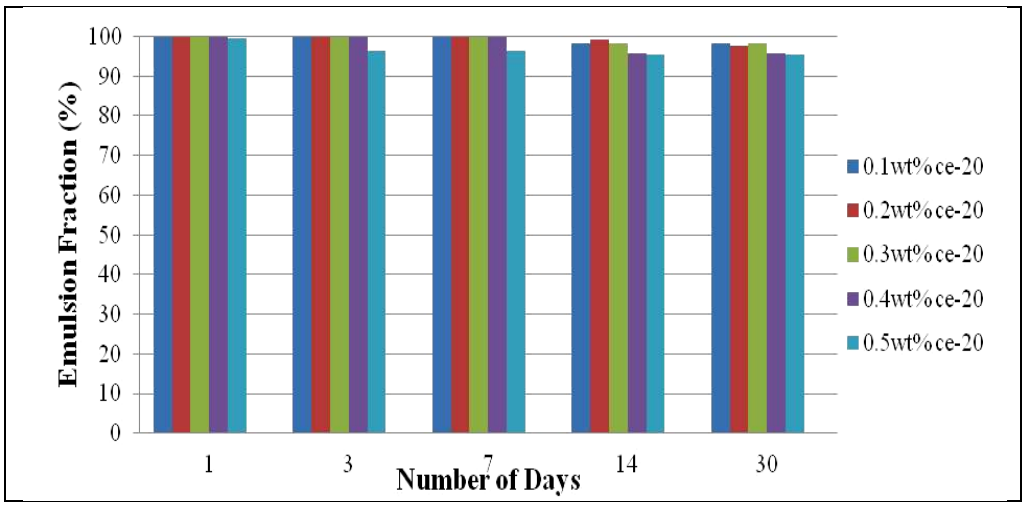


Figure 3.17: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% tea tree oil, o:w = 1:1, 0.009 wt% Triton^{TM} X-45, 0.004 wt% Triton^{TM} X-100; variation of 0.1-0.5 wt% ceteareth-20 for 5 wt% (a) SS (b) SP and (c) SM.

3.4.4 Stability Test of Citronella Essential Oil Emulsion

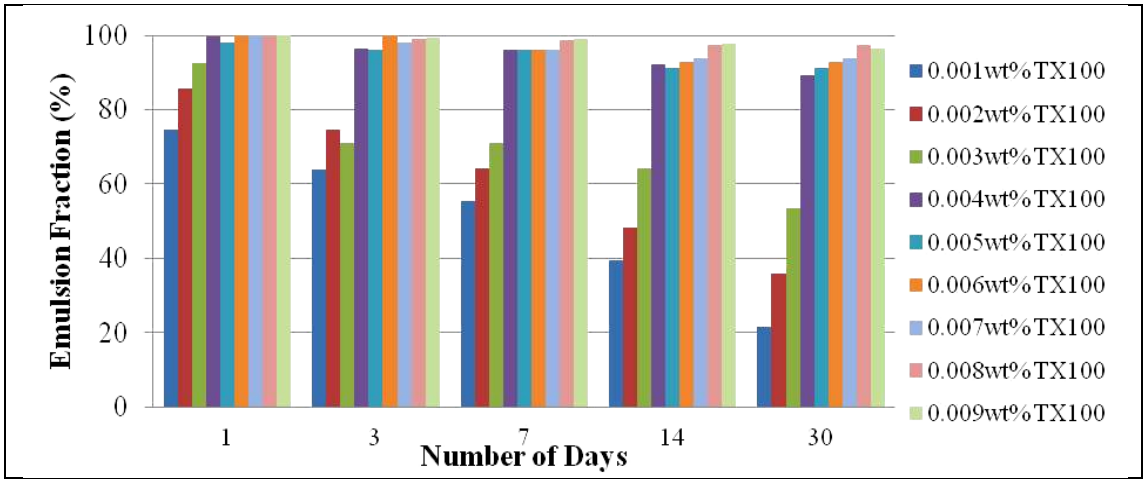
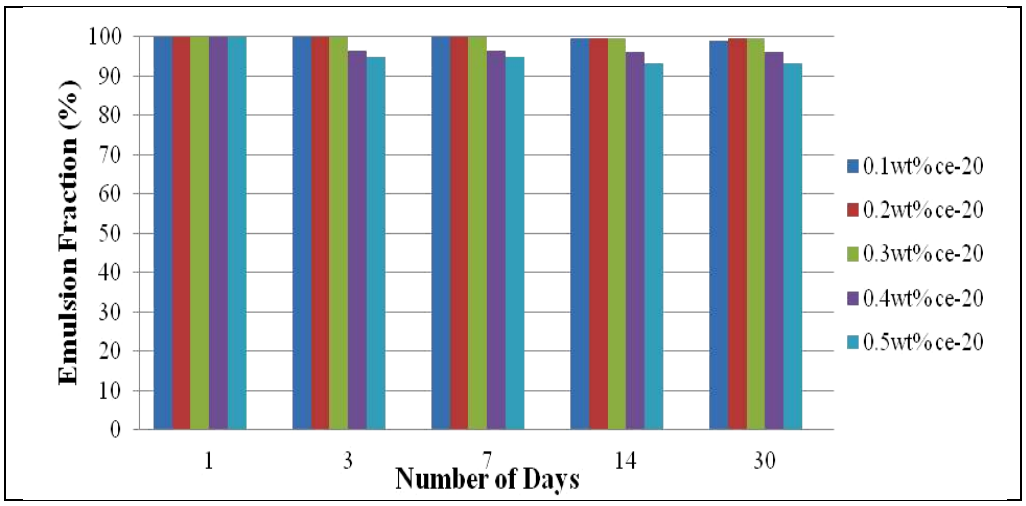


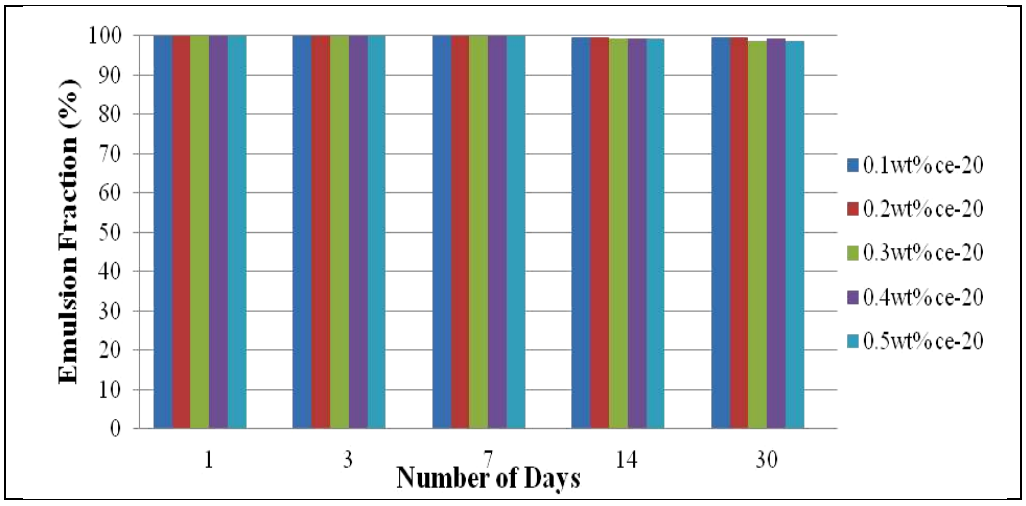
Figure 3.18: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% citronella oil, 5 wt% SP, o:w = 1:1, 0.002 wt% Triton™ X-45, 0.2 wt% ceteareth-20; variation of 0.001-0.09 wt% Triton™ X-100.

Citronella oil comprises mostly of (R)-(+)- Citronellal, which is a straight chain aldehyde with molecular formula of $C_{10}H_{18}O$ [56-60]. Unlike other essential oil, the compounds in this oil comprise of mixtures of cyclic compound, phenolic groups and benzene ring derivatives, the unique component was similar to fatty acid present in carrier oil enabling citronella oil to blend readily with carrier oil forming a stable oil phase, thus requiring the minimum amount of co-surfactant in emulsion formation. Figure 3.18 displays emulsions formation with critically low concentration of Triton™ X-45 (0.002 wt%) and Triton™ X-100 (0.001-0.009 wt%), emulsion formations are viable starting from 0.004-0.009 wt% Triton™ X-100 giving a wide range of usable parameters, typically 0.008 wt% Triton™ X-100 presenting highest emulsion fraction in the series. Ceteareth-20 used to enhance viscosity of emulsion was further reduced to 0.005-0.009 wt% (Figure 3.20) as additional ceteareth-20 (Figure 3.19) present does not facilitate emulsion stability, causing a slight phase separation of emulsion decreasing the emulsion fraction present. Therefore, formula of citronella emulsion was determined to be 12.5 wt% citronella oil, o:w = 1:1, 0.002 wt% Triton™ X-45, 0.008 wt% Triton™ X-100 and 0.006 wt% ceteareth-20.

(a)



(b)



(c)

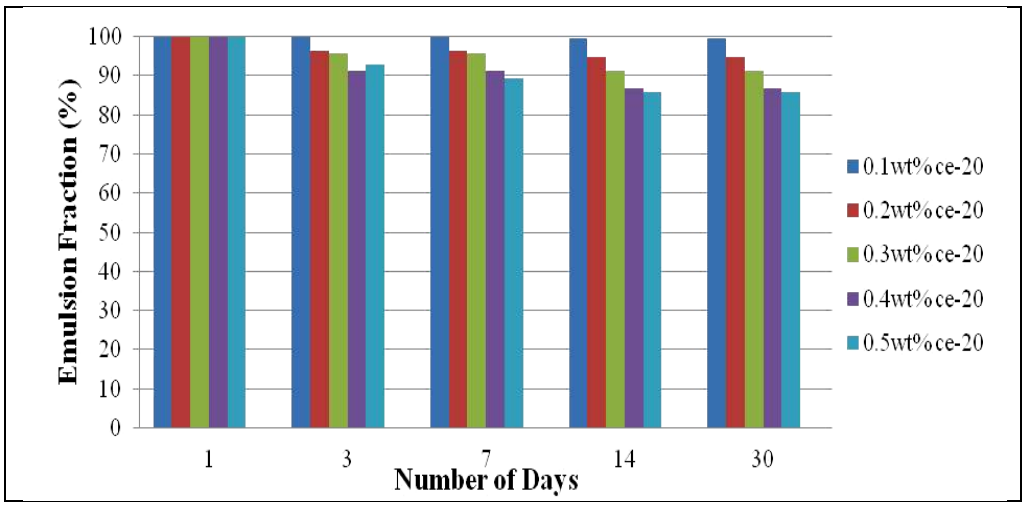
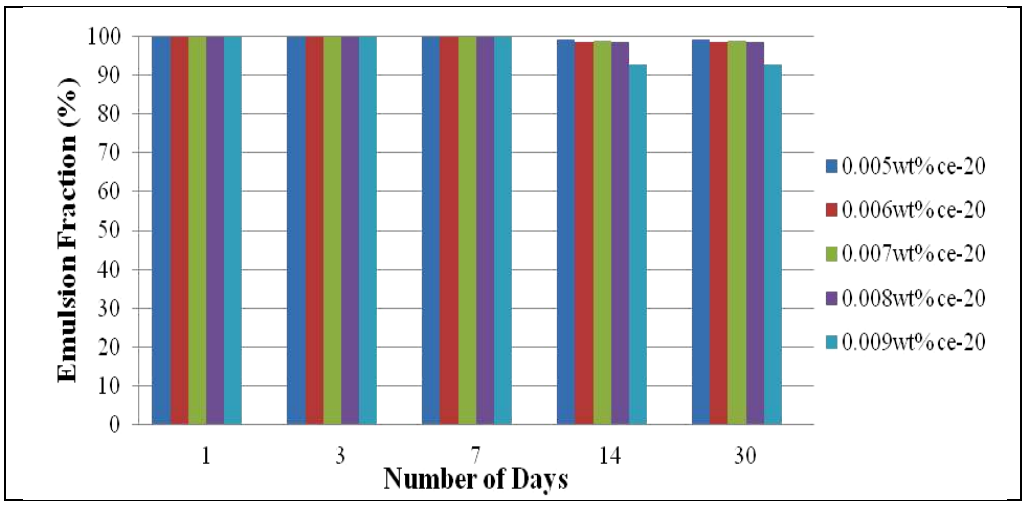
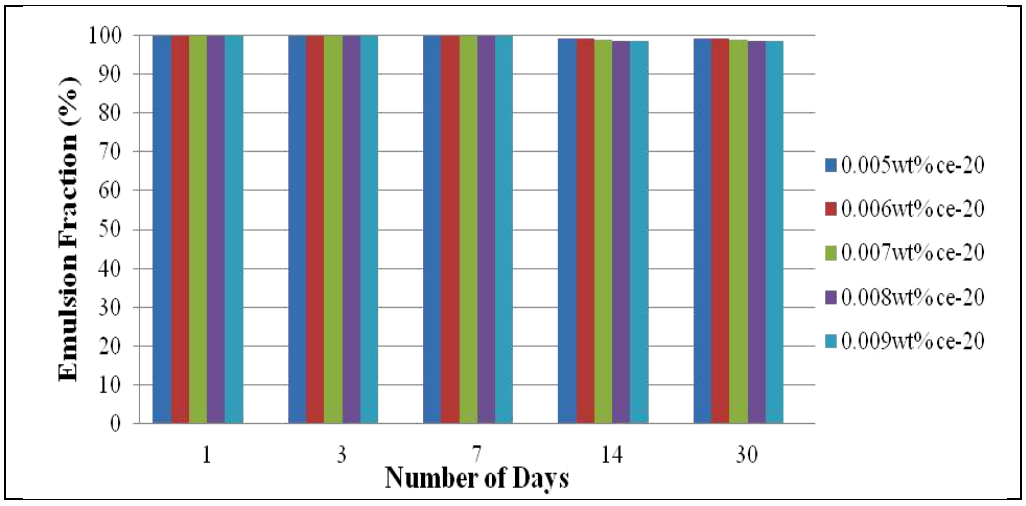


Figure 3.19: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% citronella oil, o:w = 1:1, 0.002 wt% Triton^{TM} X-45, 0.008 wt% Triton^{TM} X-100; variation of 0.1-0.5 wt% ceteareth-20 for 5 wt% (a) SS (b) SP and (c) SM.

(a)



(b)



(c)

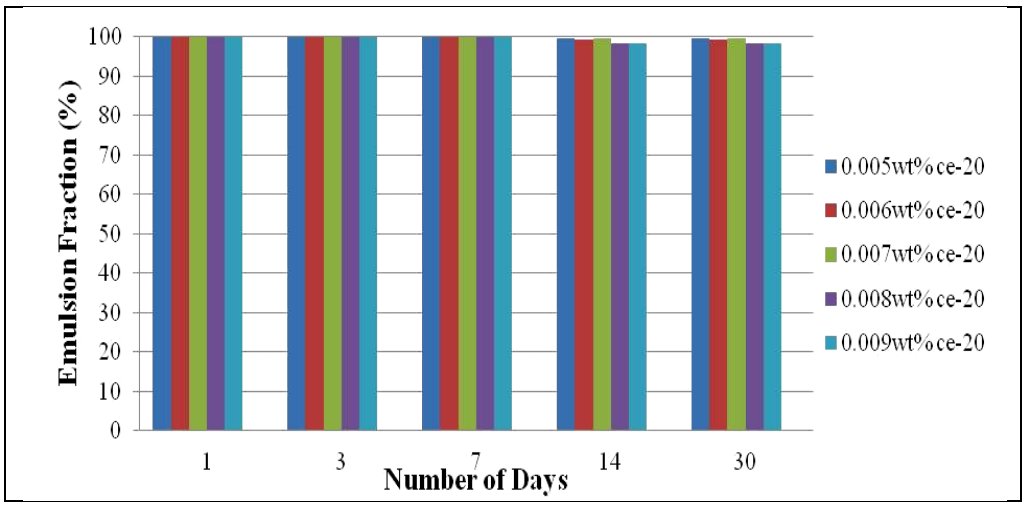


Figure 3.20: Accelerated test of emulsions at 45.0±0.1 ºC, 12.5 wt% citronella oil, o:w = 1:1, 0.002 wt% Triton™ X-45, 0.008 wt% Triton™ X-100; variation of 0.005-0.009 wt% ceteareth-20 for 5 wt% (a) SS (b) SP and (c) SM.

3.5 Light Micrograph, Mean Droplet Size and Zeta Potential Analysis of Essential Oil Emulsions

Emulsion stability was a major concern as the suspension stability exerted a great impact on the suspension ability to be practically functional and the capability to resist destabilization throughout the lifetime of the product. Emulsion systems are typical thermodynamically unstable system, the colloidal suspension undergoes separation of dispersed phase from continuous phase over a period of time through kinetic or thermodynamic destabilization [159] for example, viscosity of continuous phase govern the kinetic stability while steric and electrostatic stabilization endorsed stability through particle repulsion [144]. Therefore, a series of measurement of light micrograph analysis, mean droplet size and zeta potential had been carried out to evaluate the stability of essential oil emulsions over time.

Visual observation on emulsion morphology has been carried out by using light polarizing microscope from Leica model DM RXP capturing micrograph of essential oil emulsions as in Figure 3.21. All captured micrograph show a continuous, small droplet and polydispersed emulsions formed within essential oil emulsion system. Due to the minute droplet observed, quantitative measurement of mean droplet size by using micrograph was not feasible. Therefore, light scattering measurement of droplet size distributions was carried out [160-162] by using Malvern Zetasizer Nano ZS. The system determines size by measuring Brownian motion of particles in sample using Dynamic Light Scattering (DLS) and interpreting size from establish theories [131].

Brownian motion causes emulsion droplets to move randomly and continuously colliding with each other which increases the chance of aggregation. Since, the density between dispersed phase and continuous phase was significantly sizable, gravitational forces play an important role in determining droplet stability. A stable system would be

expected if the ratio of equation 7 was less than unity, while sedimentation would occur if the ratio was greater than unity. Van der Waals attractive forces set additional factor leading to formation of flocculates with larger droplet size which was greatly influenced by gravitational force causing settling of emulsion [131].

Ratio of gravitational to Brownian forces:

$$\frac{a^4 \Delta\rho g}{\kappa_B T} \tag{7}$$

Where a, is the parcel radius, $\Delta\rho$ is the density difference between dispersed and continuous phases, g is acceleration due to gravity, κ_B is the Boltzmann constant and T is temperature [132, 163].

Malvern Zetasizer Nano ZS was also used in the measurement of zeta potential which is the potential at the slipping plane between the particle surface and associated double layer with surrounding solvent [164, 165]. Anionic glycolipid surfactants were used in the emulsion therefore producing negatively charged zeta potential value. Droplets having greater zeta potential value will experience greater repulsion and hence decreases the tendency of droplet coming close to each other to flocculate which increases the stability of the emulsions systems [80, 99, 166].

3.5.1 Morphology, Particle Size and Zeta Potential Analysis of Thyme Essential Oil Emulsion

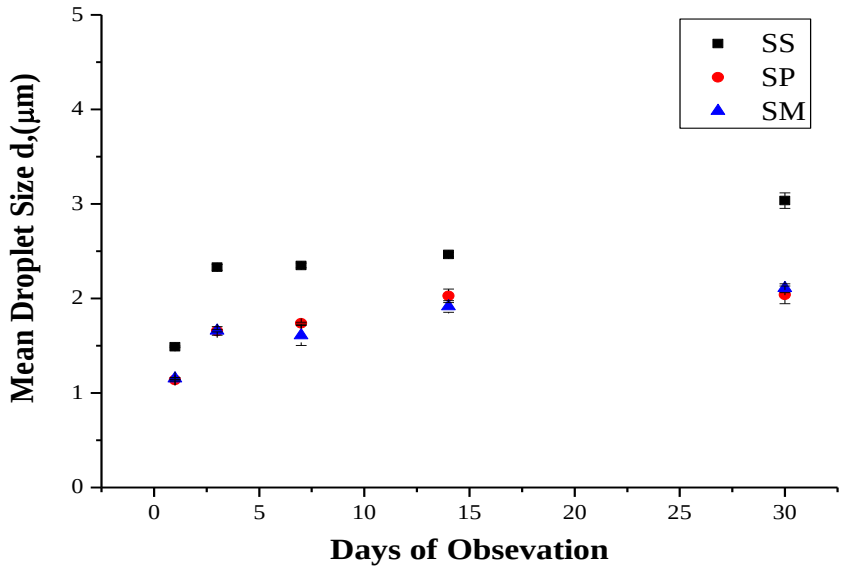
(a)

(b)

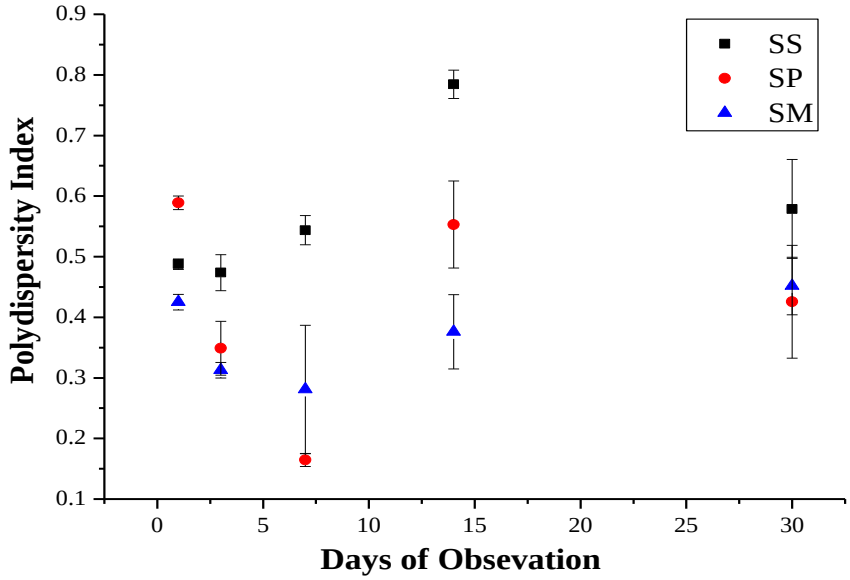
(c)

Figure 3.21: Light micrograph of thyme oil emulsions stabilized by glycolipid surfactants of (a) SS (b) SP, (c) SM. The micrograph was captured after one day of storage at temperature of 45.0±0.1 ºC.

(a)



(b)



(c)

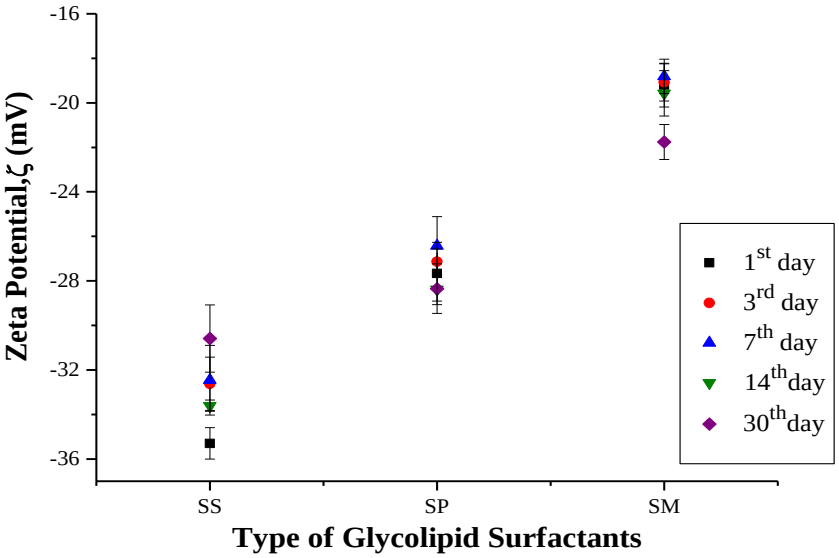


Figure 3.22: The measurements of (a) mean droplet size (b) polydispersity index and (c) zeta potential of thyme essential oil emulsions stabilized by glycolipid surfactants taken for duration of 30 days.

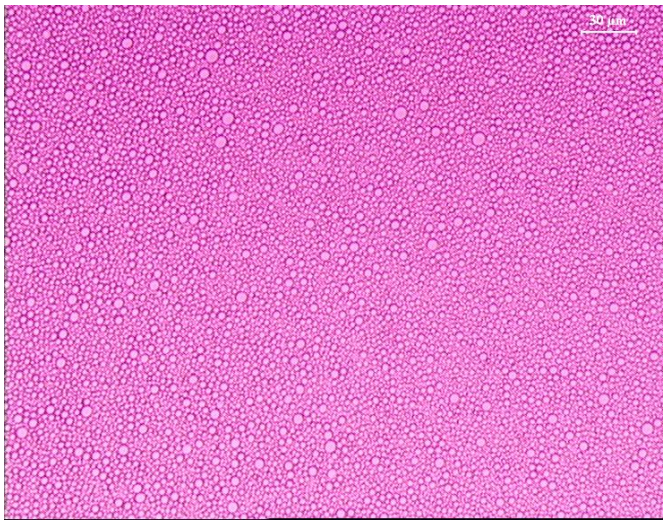
Figure 3.22 (a) shows graphical representation of mean droplet size of thyme essential oil emulsions stabilized by glycolipid surfactants with SS emulsions having the biggest mean droplet size followed by SP and SM emulsions. Due to the unequal chain length of surfactant and co-surfactant present in the emulsion system, lateral molecular interactions of van der Waals interactions was weakened as longer lipid tail of glycolipid surfactants possessed a great tendency to disrupt molecular packing with increased motion of excess fragment of lipid tail [167-169]. SS having a longer lipid tail cannot be solubilised perfectly in the oil phase driving the lipid tail proximately close to the interface and cramping the quarter increasing the inter-particle distance between surfactant which causes reduction in surface area of emulsion droplet producing a significantly bigger size droplet of emulsion as compared to shorter chain glycolipid surfactants. However, emulsion having a smaller droplet size was relatively more stable than those of larger droplet [170, 171].

As storage duration increases ageing of emulsion was observed as mean droplet size of emulsion increases with storage duration, the increase was recorded rapid for SS while SP and SM emulsions shows relatively low and less rapid increment indicating a more stabilized emulsions system than SS emulsions. Destabilization of emulsions was greatly attributed to the dynamic property at the surfactant layer, interfacial properties and colloidal interactions of emulsion [166, 172].

Meanwhile, zeta potential of emulsions stabilized by SS shows greater negative potential values than SP and SM suggesting a decreasing stability of emulsion formed according to the decrease in lipid tail length. SS having a biggest droplet size was stabilized by higher zeta potential value repulsing droplet from emerging opposing destabilization through flocculation. As for emulsion stabilized by SM, zeta potential value of emulsion formed was recorded to be the lowest suggesting low inter-particle repulsion leading to flocculation and destabilization over a long storage period.

3.5.2 Morphology, Particle Size and Zeta Potential Analysis of Lavender Essential Oil Emulsion

(a)



(b)

(c)

Figure 3.23: Light micrograph of lavender oil emulsion stabilized by glycolipid surfactants of (a) SS (b) SP, (c) SM. The micrograph was captured after one day of storage at temperature of 45.0±0.1 ºC.

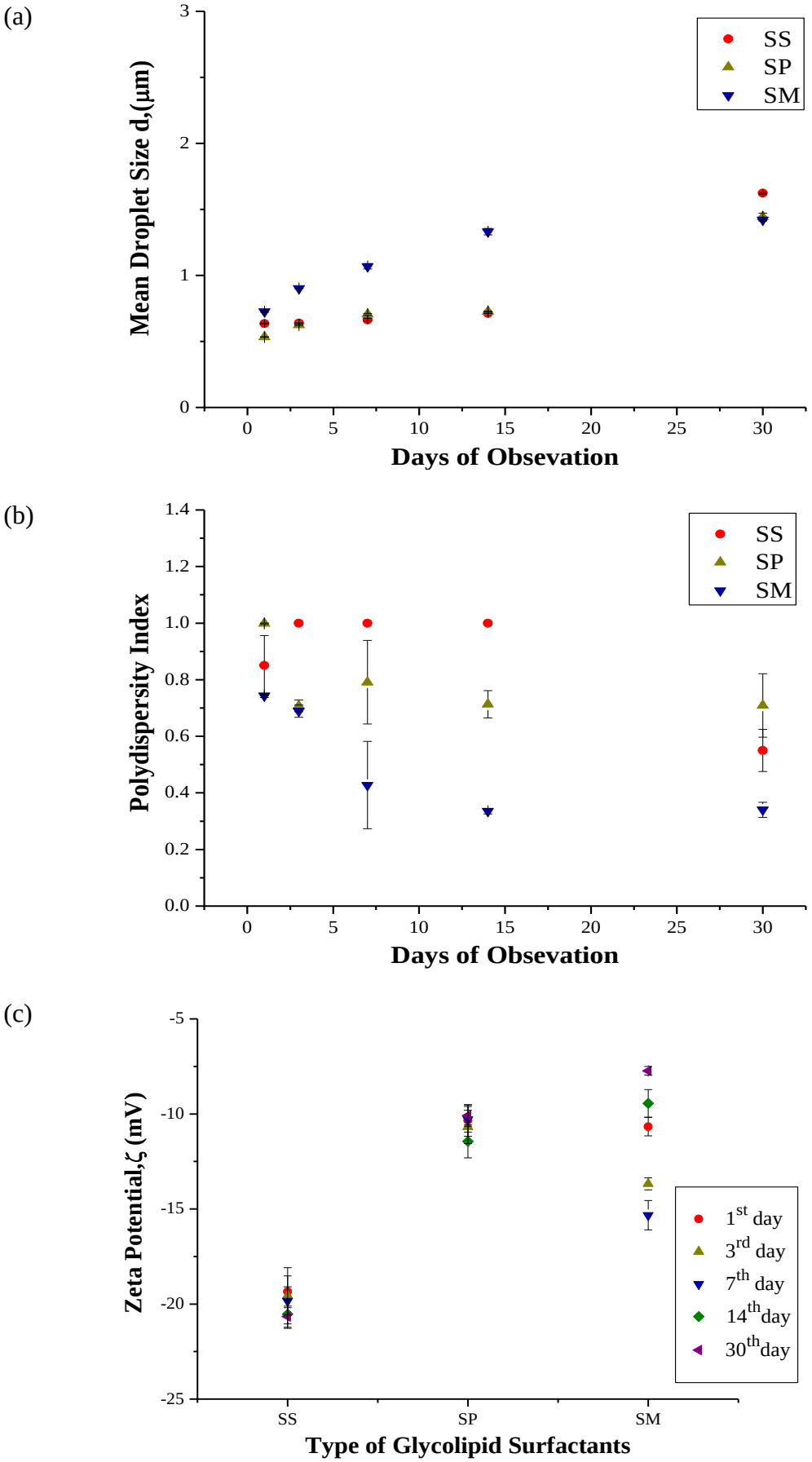


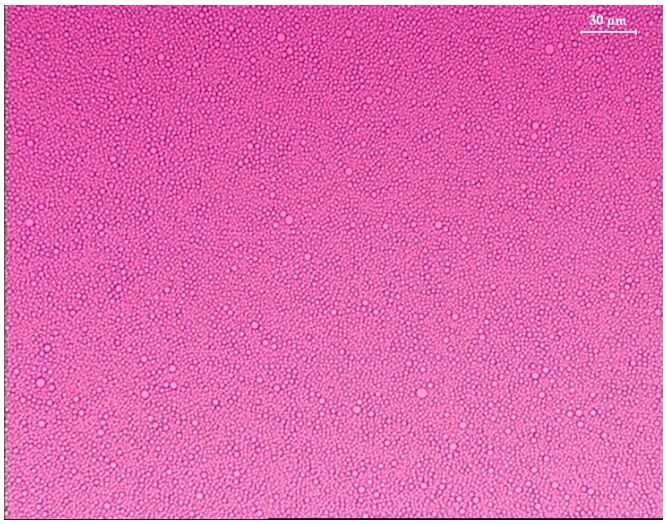
Figure 3.24: The measurements of (a) mean droplet size (b) polydispersity index and (c) zeta potential of lavender essential oil emulsions stabilized by glycolipid surfactant taken for duration of 30 days.

Essential oil compositions determined the ability of formation of droplet with association to the inter-particle forces and chemical structures present. Formations of smaller droplet caused by straight chain terpenes and terpenoids in lavender oil generate lower steric repulsion in the bulk phase causing shrinking of mean droplet size of emulsions [135, 163]. Formation of emulsions droplet not abiding to the characteristic flow with variation of droplet size and uneven distributions were observed for lavender emulsion. The mean droplet sizes of lavender emulsions stabilized by SP were recorded bigger than SS and SM emulsions as in Figure 3.24 (a). Distribution of means droplet size with variation of PDI resulted in increase of viscosity from SS followed by SM and finally SP emulsions.

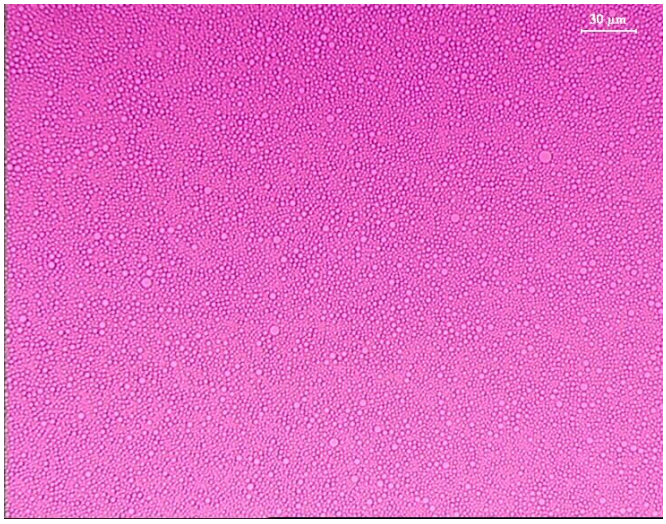
As storage duration increases, the mean droplet size of SP and SM emulsion increases rapidly as ageing took place. Weak inter-particle association corresponding to decreasing zeta potential and PDI lead to weak integrity between droplets which mark the dissociation and instability of emulsions system formed for both SP and SM emulsions after 14^{th} days of observation. However, SS emulsion with lower fluctuations of droplet size, steady zeta potential values retains the stability of lavender emulsions over a period of 30 days.

3.5.3 Morphology, Particle Size and Zeta Potential Analysis of Tea Tree Essential Oil Emulsion

(a)



(b)



(c)

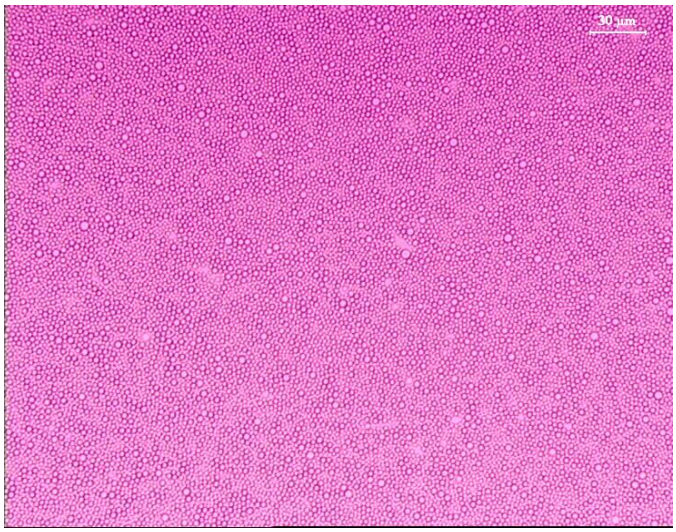
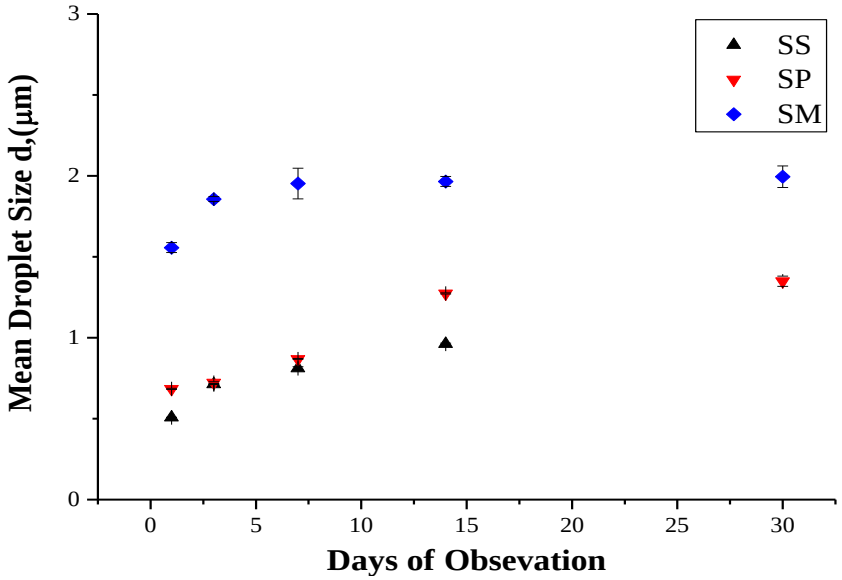
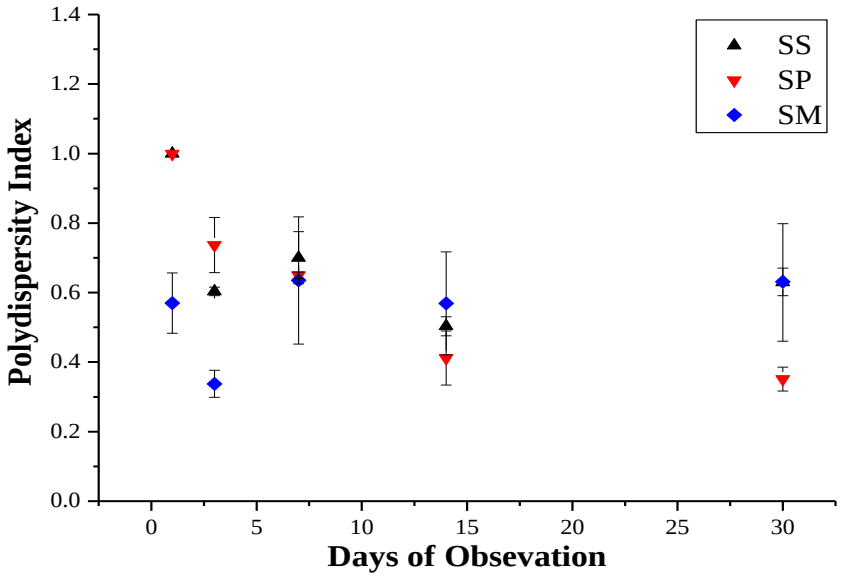


Figure 3.25: Light micrograph of tea tree oil emulsion stabilized by glycolipid surfactants of (a) SS (b) SP, (c) SM. The micrograph was captured after one day of storage at temperature of 45.0±0.1 ºC.

(a)



(b)



(c)

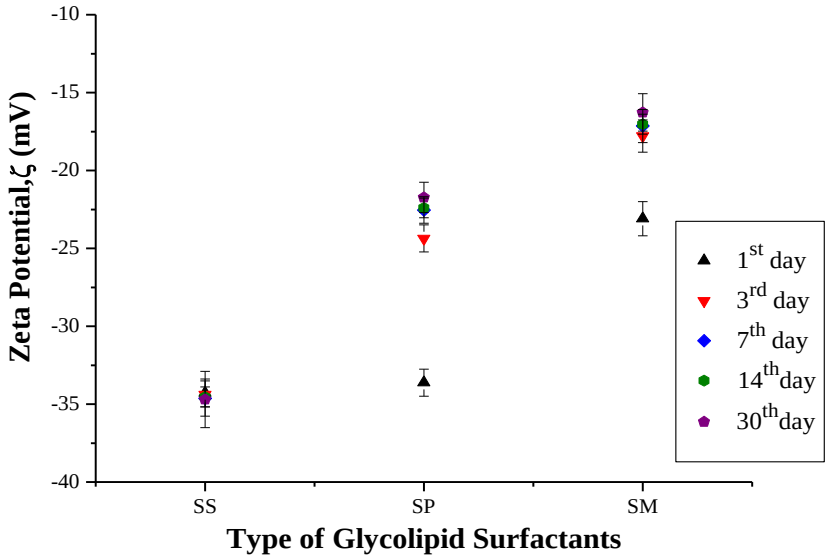


Figure 3.26: The measurements of (a) mean droplet size (b) polydispersity index and (c) zeta potential of tea tree essential oil emulsions taken for duration of 30 days.

The mean droplet size of tea tree oil emulsions as displayed in Figure 3.26 (a) does not obey similar trend of the thyme oil, lavender oil and citronella oil emulsions mean droplet size profile. The data recorded a bigger mean droplet size of SM emulsions as compared to the SP and SS emulsions. The ceteareth-20 acting as the thickening agent has participated in the formations of emulsion droplets stabilizing the systems and causes a bigger size droplet formed.

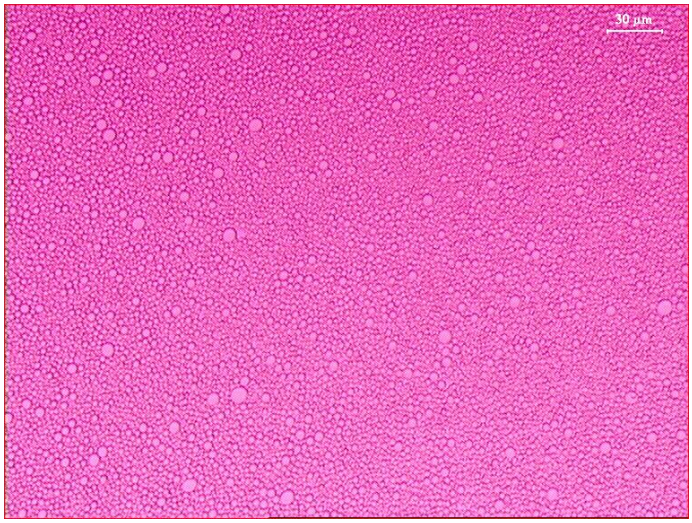
The phenomenon observed decreases the concentration of ceteareth-20 in the exterior reservoir which hinders the formation of weak gel structure withholding the emulsions droplet and hence causes the extraordinarily low viscosity recorded. In addition, low zeta potential value of SM emulsions causes the reduce in slip resistance between emulsions layer results in a low inter-particle repulsions which further reduce the stability and viscosity of SM emulsions [131].

As storage duration increases, ageing of emulsions were observed as the mean droplet size of emulsions increases for all the emulsion system tested. The overall increase was not rapid, but due to the reduced amount of emulsion fractions in SM emulsions signifying dissociation of emulsions, the emulsions were concluded to be of the least stable. On the other hand, tea tree emulsions stabilized by SS which displayed smaller mean droplet size and higher zeta potential values suggested a more stabilized emulsion system than SP and SM emulsions.

3.5.4 Morphology, Particle Size and Zeta Potential Analysis of Citronella Essential Oil Emulsion

(a)

(b)



(c)

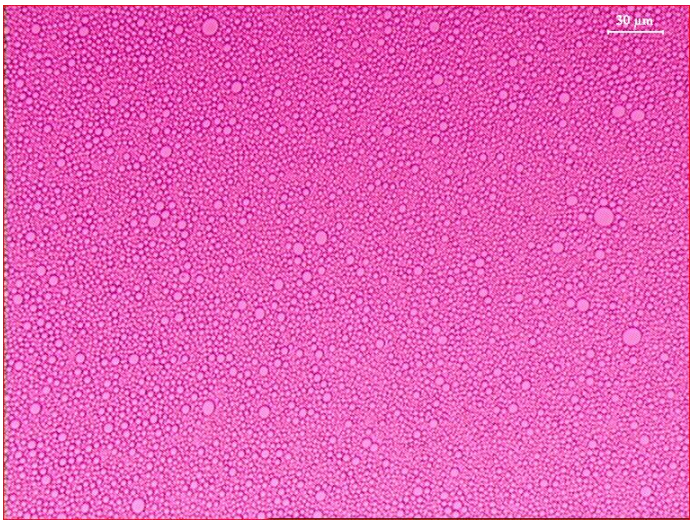


Figure 3.27: Light micrograph of citronella oil emulsion stabilized by glycolipid surfactants of (a) SS (b) SP, (c) SM. The micrograph was captured after one day of storage at temperature of 45.0 ± 0.1 ºC.

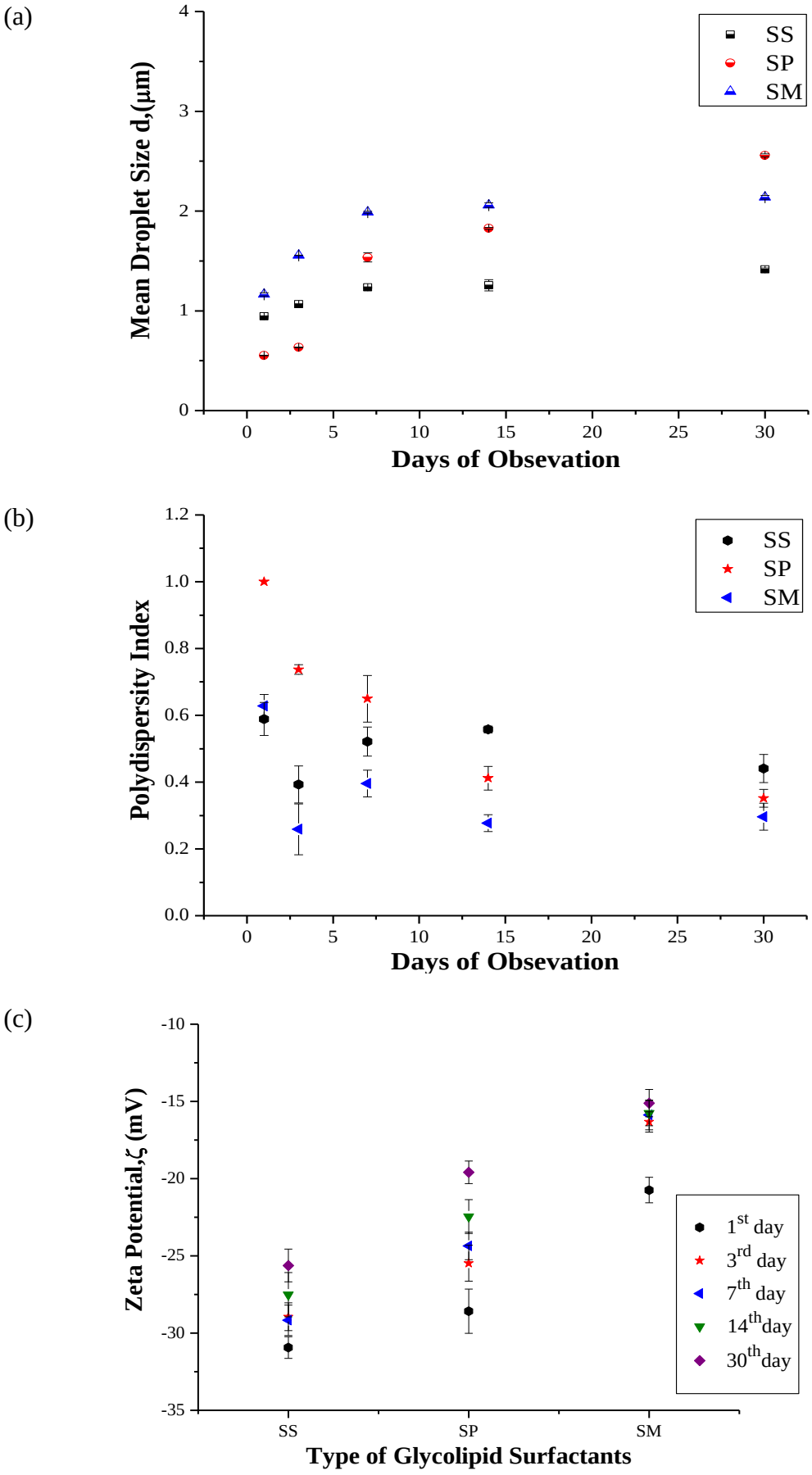


Figure 3.28: The measurements of (a) mean droplet size (b) polydispersity index and (c) zeta potential of citronella essential oil emulsions stabilized by glycolipid surfactants taken for duration of 30 days.

Citronella oil emulsions shows relatively comparable small mean droplet size for all glycolipid emulsions studied as shown in Figure 3.28 (a). The accelerated ageing process causes rapid increment of mean droplet size for both SP and SM emulsions, while SS emulsions recorded minor increase over a period of 30 days. Generally, changes in droplet size measurably describe the stability conditions of emulsions, where rapid fluctuations of droplet size associated to dynamic changes in the internal environment were factors causing dissociations of emulsions. Whereas, zeta potential of emulsions opposes emulsion droplets from coming close and merge into bigger droplets [131, 132]. Therefore, SS emulsions with higher zeta potential shows a smaller degree of increment in the mean droplet size. Whereas, SM emulsion with lower zeta potential magnitude experiences a rapid increase of mean droplet size caused by the inability to oppose emergence of droplet due to the low repulsion energy [133].

Clearly, SP and SM emulsions were less stable as compared to SS emulsions. Two case scenarios took part in the formation of emulsions; firstly, ceteareth-20 acting as a thickener was suspended in the continuous phase entangling emulsion droplets opposing deformation. Secondly, ceteareth-20 was displaced into the bulk phase of SP and SM emulsion droplet, taking part in the formation of droplet due to the incapability the of shorter lipid tail of SP and SM of forming stabilized droplet with citronella oil. Hence, viscosity and storage energy of SS emulsions were recorded higher than SP and SM emulsions. Further addition of ceteareth-20 in formulation of emulsions does not promote stability of emulsion formed and ironically causes rupturing of emulsion droplet due to the presence of excessive amount of co-surfactant in the bulk moiety exerting forces sufficiently high to break emulsion droplets. To overcome this problem, thickeners such as polysaccharides acting by adsorbing to the surface of emulsion droplet can be added to increase viscosity and enhance stability of the emulsion formed [173].

3.6 Rheology

Rheological analysis was carried out to elucidate the insight of emulsions texture, spreading and characterize cosmetic products into respective range such as creams, lotion and gels [132]. Rheological measurements were conducted by utilizing Bohlin CVO-R Rheometer Germini^{TM} at a regulated temperature of 30.0±0.1 °C.

3.6.1 Rheological Analysis of Thyme Essential Oil Emulsions

Figure 3.29 displays the descending viscosity magnitude in the order from SS, SP and followed by SM emulsions. All emulsions exhibit shear thinning behaviour with a specific yield stress value. As shear stress increases with shear rate without any deformation until attaining a maximum value (yield stress) sufficiently high to overcome resistance of droplet packing and starts to flow as shear thinning takes an ellipsoidal shape aligned with the direction of flow causing a sharp decrease in viscosity of emulsions [174-176].

Viscosity is highly affected by mean droplet size, polydispersity index (PDI) of emulsion droplets, presences of additives, surfactant concentration, oil concentration and ageing of emulsion [177-179]. Viscosity also showed dependency on the inter-particle cohesive force arising from frictions between emulsion droplets.

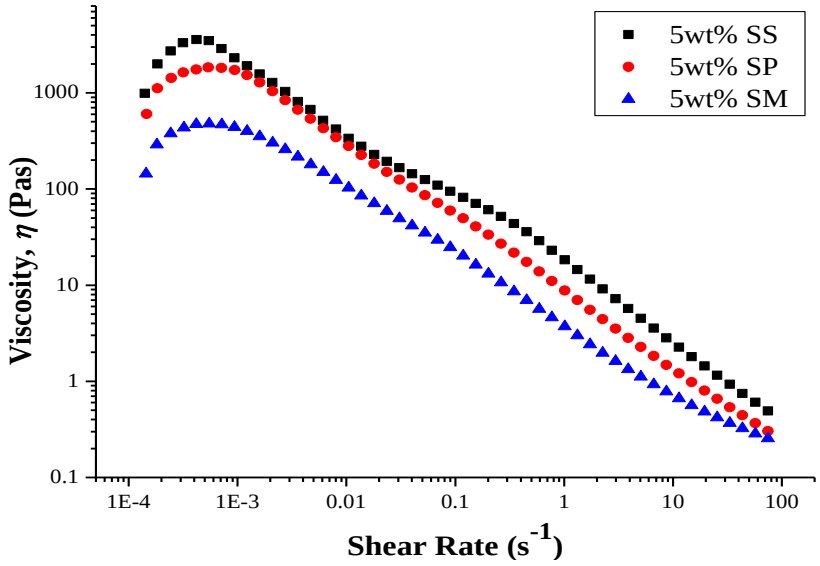
$$E_c = \frac{G'\gamma_c^2}{2} \tag{8}$$

Inter-particle interactions can be represented as cohesive force, E_c which can be estimated using storage modulus, G' and γ_c of emulsion according to equation 8. Cohesive energy is highly related to structure of emulsion system which correlates with droplet size and number of contact area between the droplets [180]. Larger cohesive interaction results in higher viscosity of emulsions.

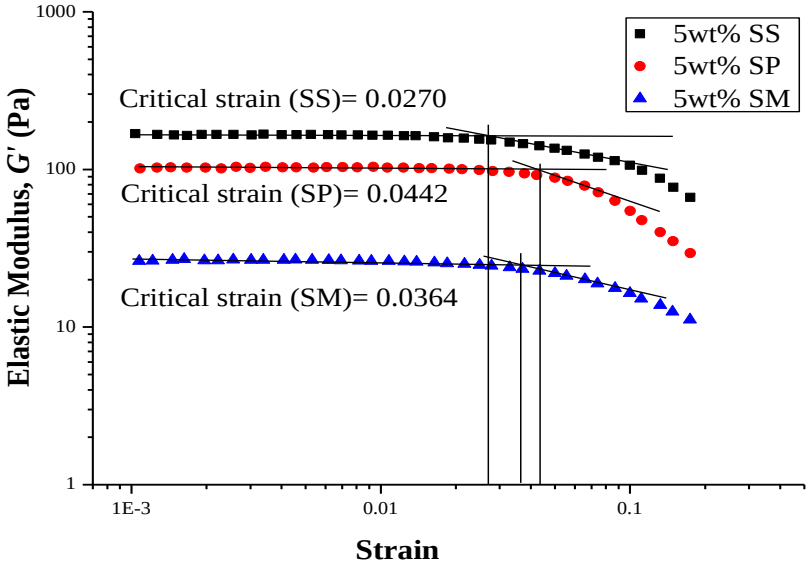
Emulsion system having bigger mean droplet size and low PDI would yield a higher viscosity due to higher resistance towards motions when larger droplets were distributed evenly in a system [175]. Since mean droplet size of SS emulsion was recorded to be the highest, the corresponding viscosity was recorded to be the highest among emulsion system studied. SP and SM emulsions which possessed nearly equal mean droplet size were distinguished by the polydispersity distributions of emulsion droplets [175, 176]. SP emulsion with higher PDI consists of mixtures of droplet with variable sizes predominantly small size droplets causing them to possess a lower viscosity than SS emulsions but higher viscosity as compared to SM emulsion. Emulsion stabilized by SM records the lowest mean droplet size with lowest PDI illustrating emulsions to be distributed uniformly with small size droplet resulting in low viscosity emulsion system [175].

Figure 3.29 (b) displays amplitude sweep or strain test, preformed by increasing amplitude with constant temperature of 30.0±0.1 °C and frequency of 0.5 Hz. *G'* (storage modulus) and *G"* (loss modulus) responses nearly parallel at low strain giving a LVR until *G'* declined and being dominated by *G"* where sample undergoing deformation starts to flow [181]. Results show that the *G'* value decreases from SS, SP and finally SM emulsion. SS emulsion was stiff due to high *G'* [180], but having lowest LVR and lowest critical strain of 0.0270 unit suggesting weak emulsion integrity as they have lowest resistivity towards deformations [174]. Though emulsion stabilized by SP was less stiff due to lower *G*' value, but they retain high structural integrity due to high critical strain and long LVR. Possessing the lowest *G'*, emulsion stabilized by SM was softest and weakest among the emulsion studied.

(a)



(b)



(c)

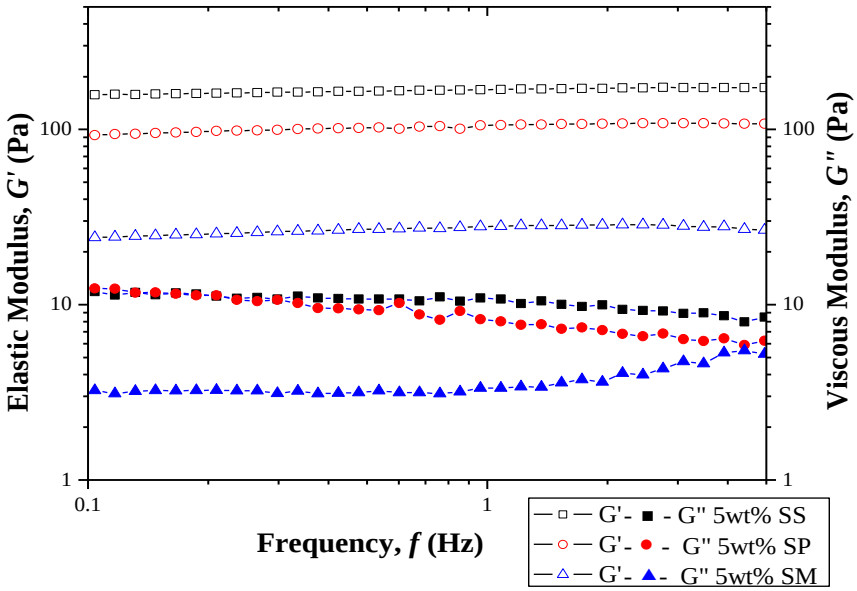


Figure 3.29: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of thyme emulsion stabilized by glycolipid surfactants on first day of observation.

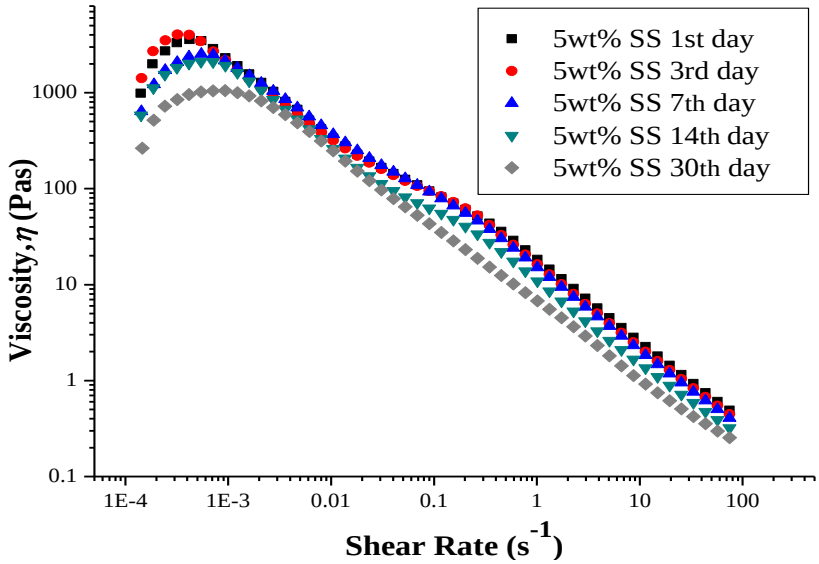
Figure 3.29 (c) displays the frequency dependent oscillatory measurements of essential oil emulsions with *G'* predominates *G"* modulus by a factor of 10 responded almost parallel throughout the measured frequency (ω) domain implying solid like behaviour of emulsion [182] signifying great internal strength with high inter-particle interactions forces between droplets. SS and SP emulsions having a close pack rigid structure where droplets resist movement with increasing frequency giving relatively straight *G'* response. SM emulsion shows lowest *G'* and *G"* due to smaller interacting internal forces caused by internal small size droplet distribution which reduces the packing rigidity [180]. As frequency increases, loss of elasticity was observed for SM emulsions with decreasing *G*' at high frequency. Therefore, SS emulsion endorsed the most rigid structure representing solid like behaviour followed by SP and SM emulsions. By considering the flow behaviour, LVR, mean droplet size and stability of emulsions, essential oil emulsion formed from SP was concluded to be of stable, having intermediate viscosity and gives a wide range of application with long LVR.

Measurements are repeated at discrete time intervals at 1^{st}, 3^{rd}, 7^{th}, 14^{th} and 30^{th} days (Figure 3.30-3.32) with constant temperature and measurement parameters. Comparison has been made to determine the changes of internal structure to determine product stability over a long shelf life. Viscosity decreases over the period of test, emulsions are subjected to internal structure rearrangement and destabilization processes namely coalescence and flocculation [165]. Mean droplet size and PDI of emulsions increase with period of test as ageing takes place. PDI increases more rapidly than the mean droplet size of emulsion signifying a more polydispersed system, which reduces the packing ability of droplet and lowering structural integrity which reduces *G'* of the system resulting in the decline of inter-particle cohesive forces (equation 8) leading to a decrease in viscosity of emulsion over the period of 30 days. Presence of inter-particle hydrogen bonding records higher storage energy of thyme emulsion than

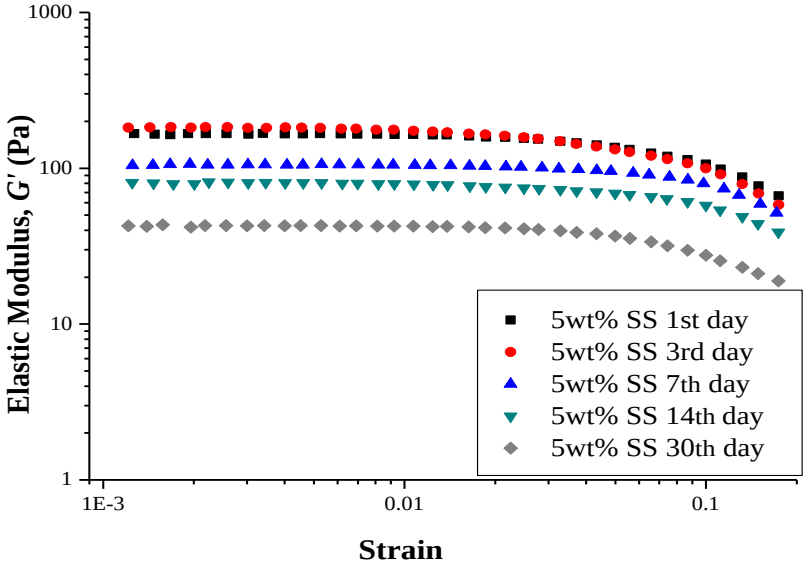
essential oil held with weaker van der Waals forces [102]. In contradiction, presence of inter-particle hydrogen bonding increases the merging rate of emulsion droplet causing formation of droplet with bigger size and more rapidly as compared to other essential oil emulsions system [109].

Amplitude dependent and frequency dependent oscillatory measurements yield similar profile to the initial measurements with decreasing gap between *G'* and *G"* moduli for all thyme emulsions, indicating decreasing structural integrity resulting in loss of elasticity at high frequency [132]. Both profile and critical strains are in matching number signifying low structural rearrangement giving a potentially stable emulsion system resisting internal structure rearrangement with ageing [163]. Overall decrease does not cause major dissociation of internal structure which would possibly lead to separations into respective phases. Therefore, thyme emulsions are concluded to be stable as they withheld the ability to maintain high emulsion fraction over 30 days of test resisting major changes in the internal structure.

(a)



(b)



(c)

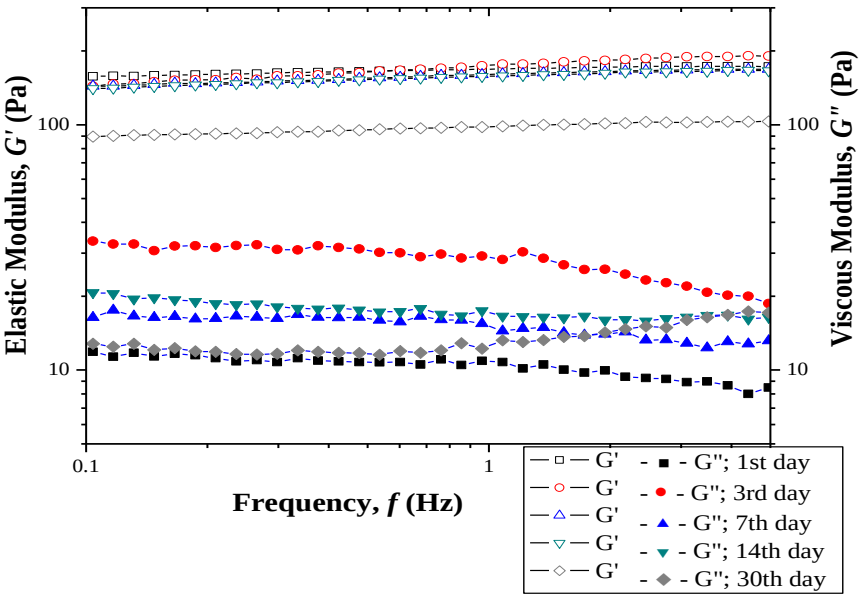
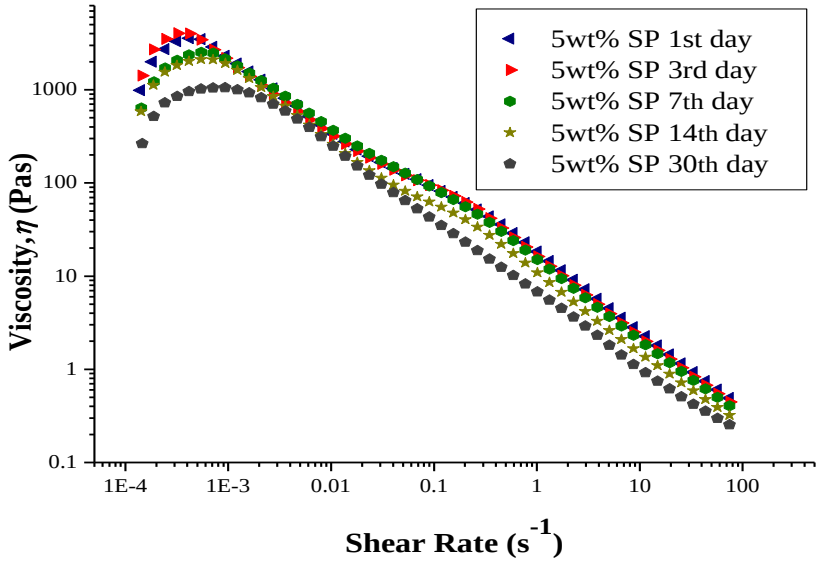
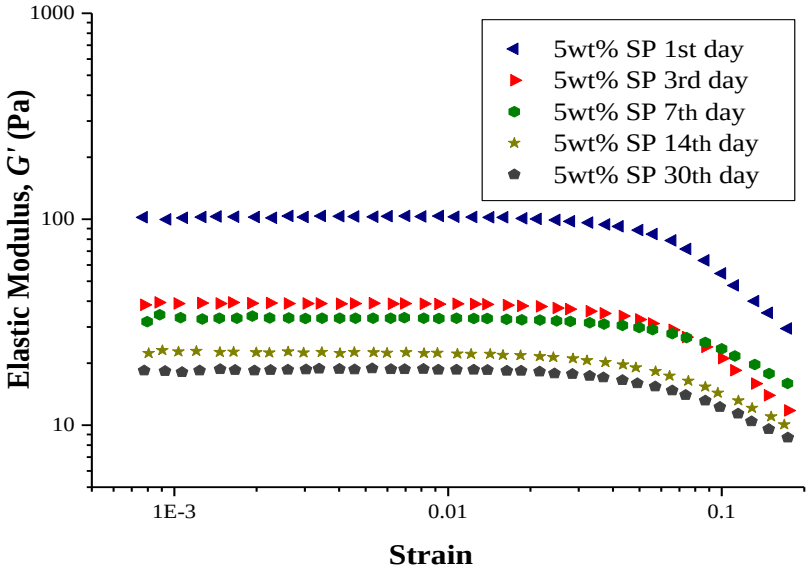


Figure 3.30: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of thyme emulsion stabilized by sucrose stearate after 30 days.

(a)



(b)



(c)

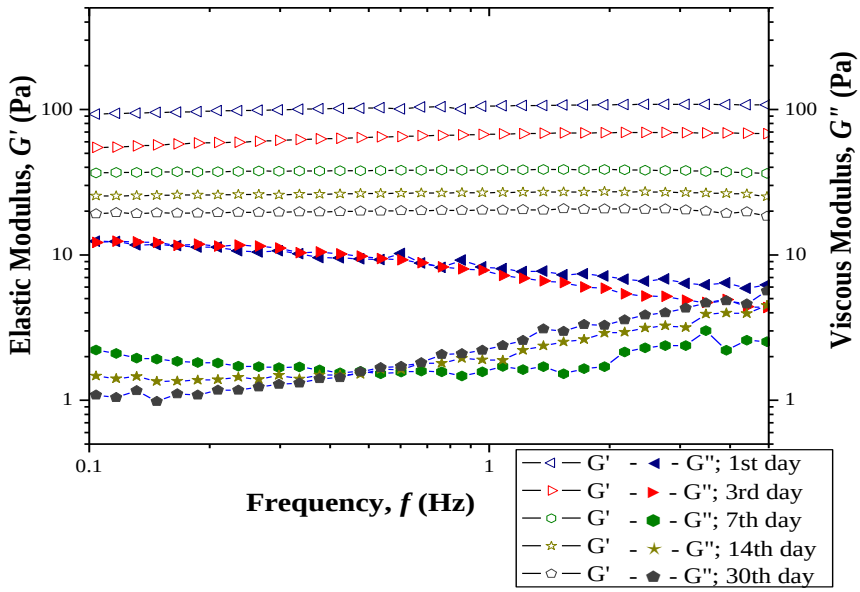
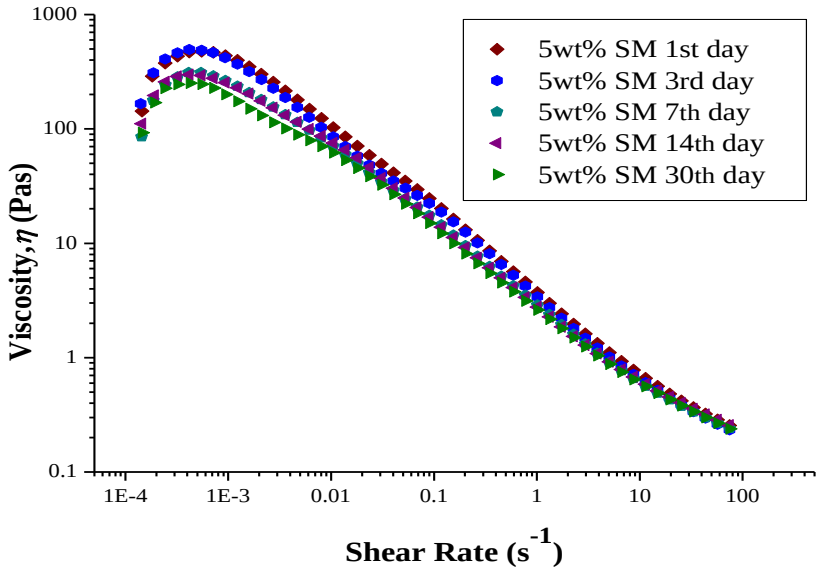
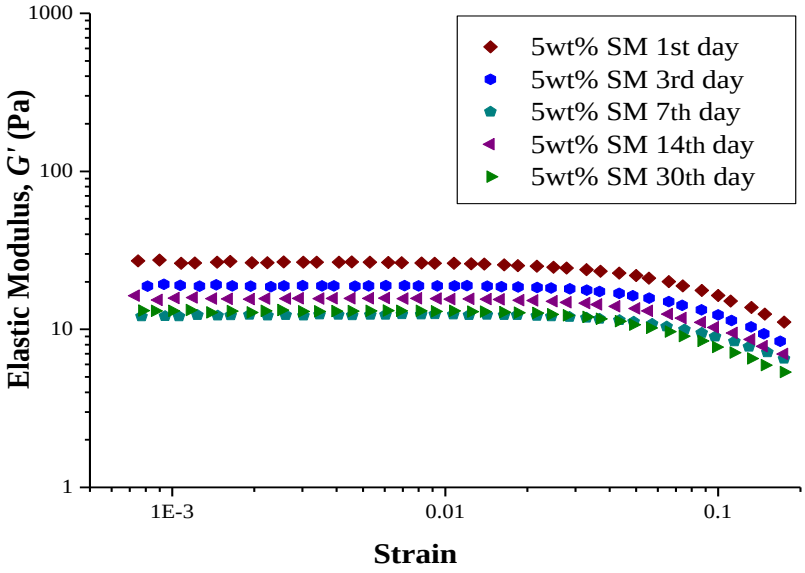


Figure 3.31: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of thyme emulsion stabilized by sucrose palmitate after 30 days.

(a)



(b)



(c)

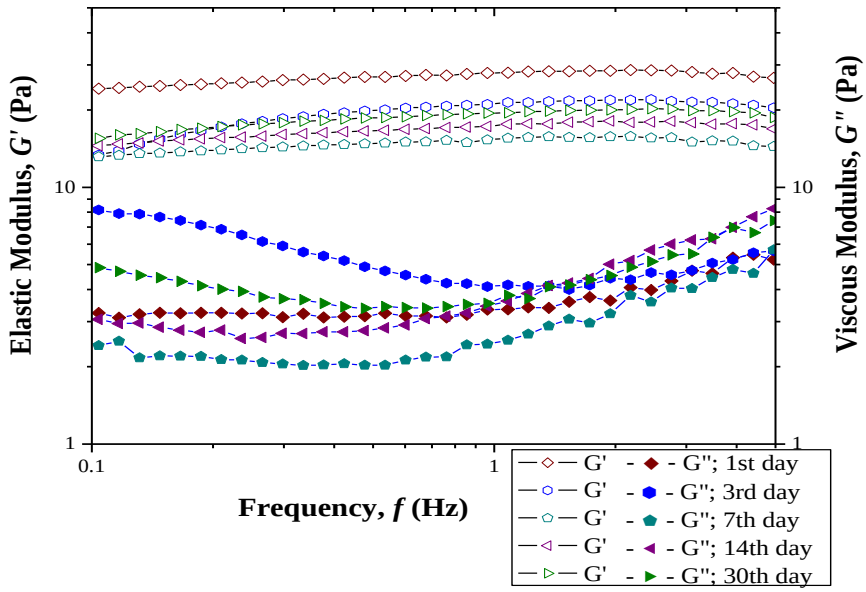


Figure 3.32: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of thyme emulsion stabilized by sucrose myristate after 30 days.

3.6.2 Rheological Analysis of Lavender Essential Oil Emulsions

Figure 3.33 (a) showed viscosity profile for lavender oil emulsions stabilized by glycolipid surfactant with similar viscosity profile for SM and SP emulsions and slightly lower viscosity recorded for SS emulsions. The smaller size emulsions droplets of SS emulsions with higher PDI causes the reduction in viscosity of SS emulsions as compared to SM and SP emulsions. Cohesive forces between droplets were predicted to be the lowest for SS emulsions due to high polydispersity distribution of droplet causing low packing ability of emulsions resulting in lower *G'*.

This was further verified by the frequency dependent oscillation profile that displays a narrow gap between *G'* and *G"* at low frequency and decreasing *G"* at high frequency indicates moderate structural strength withholding the droplet promoting flow at high frequency. Data presented show a similar profile for amplitude and frequency sweep for all lavender emulsions, Figure 3.33 (b) and (c). Critical strain values were recorded as 0.0258, 0.0465 and 0.0415 units for SS, SP and SM emulsion respectively. Despite having variation of critical strain, magnitude of approximately 100 Pa of *G'* was quantified for all lavender emulsions, indicating moderate strength internal network retaining the ability to oppose sinusoidal deformation [132, 163]. However, due to the lower *G'* magnitude, lavender oil emulsions were expected to possess a weaker inter-particle integrity and lower storage stability than thyme emulsions [172, 183].

Figure 3.34 (a) displayed the maturity of lavender oil emulsion system with increasing viscosity of the emulsions from 1st day to 7th day caused by formation of bigger emulsion droplet as in Figure 3.34 (a) and 3.35(a) followed by decreasing viscosity from 14th day onwards as the integrity holding the emulsions droplet became weaker with reduced zeta potential.

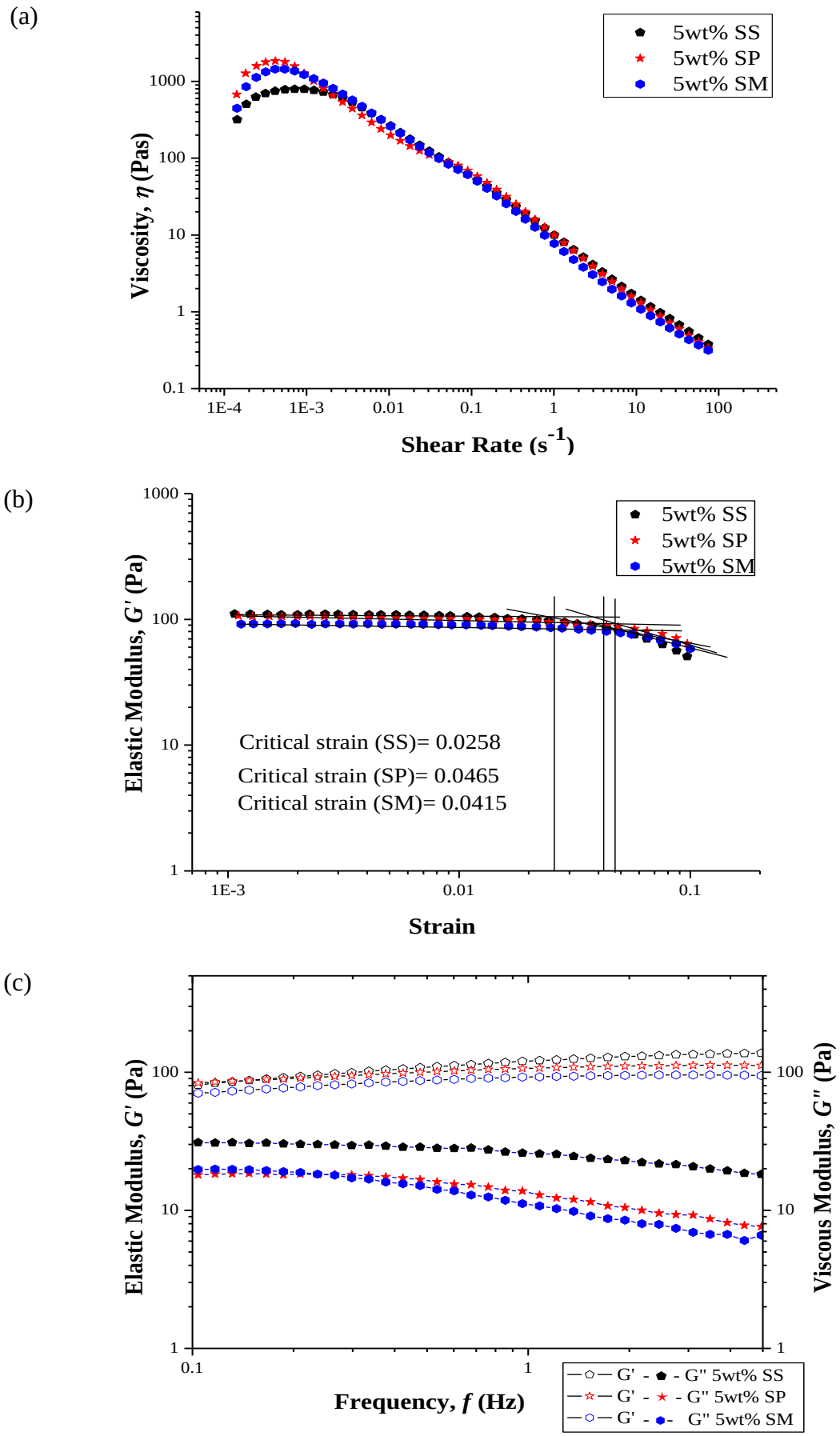
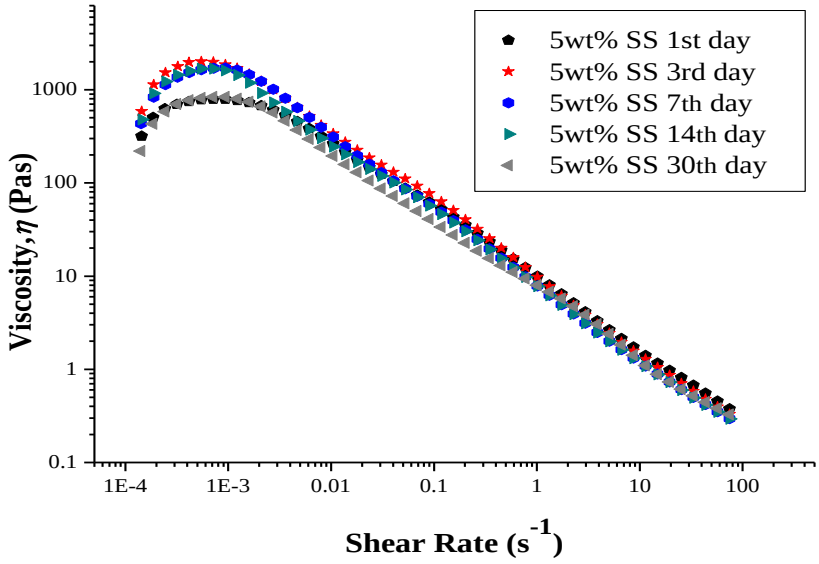


Figure 3.33: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of lavender emulsion stabilized by glycolipid surfactants on first day of observation.

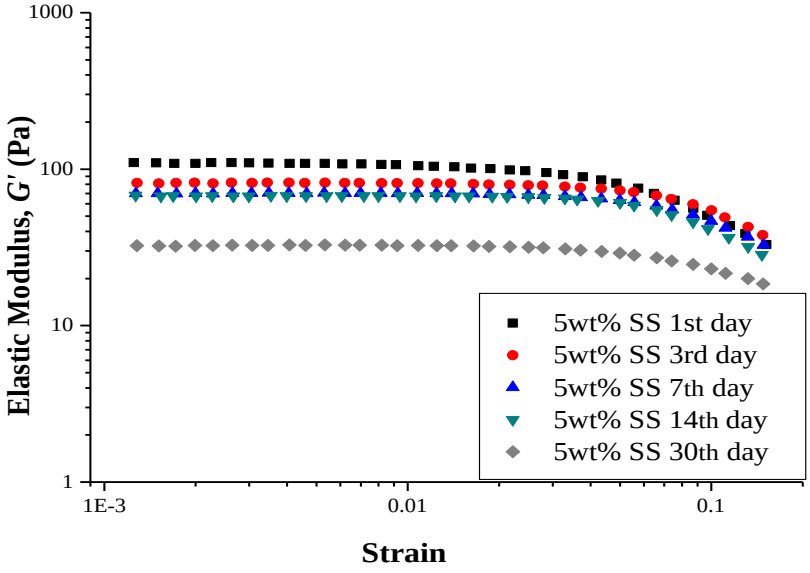
The decrease in structural integrity of the emulsions system causes the reduction in *G'* over the storage period of 30 days as shown in the strain test as in Figure 3.34 (b), 3.35 (b) and 3.36 (b), overall affecting the stability of emulsions which causes slight separation of SP and SM emulsions in the end of the test. Figure 3.35 (c) and 3.36 (c) show the double logarithmic plot against frequency for both SP and SM emulsions presents wide gap near to parallel *G'* and *G"* moduli respond indicating strong structural integrity of emulsions at the early stage of formations [164]. However, the network structure of co-surfactant ceterate-20 withholding the emulsion systems were weaken as storage period increases resulting inability of emulsion to withstand the applied stress and to deform. The phenomenon was proven by the narrowing of gap between *G'* and *G"* observed at 14^{th} days which indicates decrease in structural integrity of the system [164].

Relatively, lavender oil emulsions possessed weaker rheological properties as compared to thyme emulsions. In spite of that, lavender oil emulsions stabilized by glycolipid surfactants were able to retain high emulsions fractions over 30 days especially emulsions stabilized by sucrose stearate.

(a)



(b)



(c)

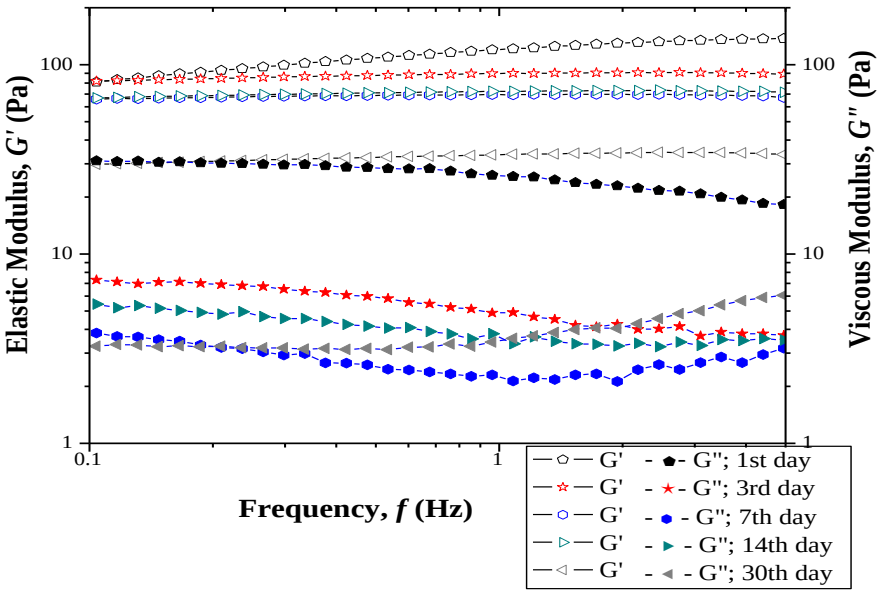
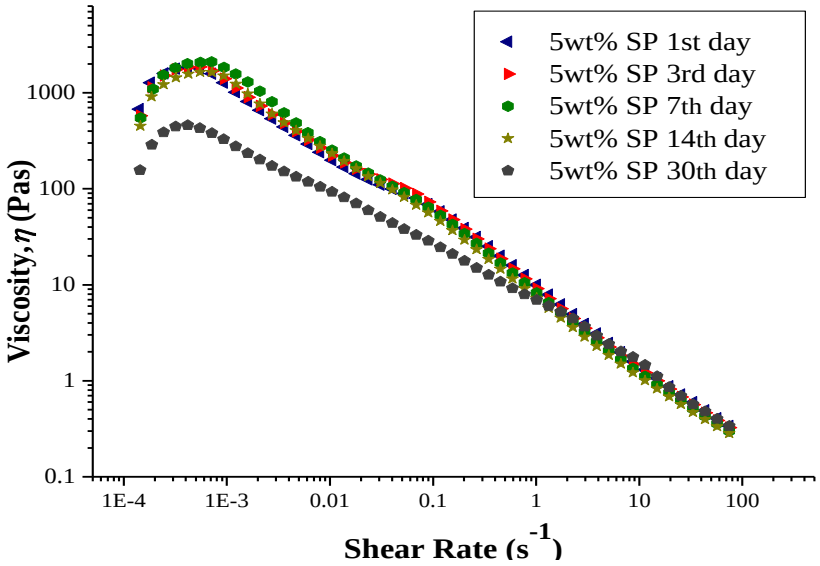
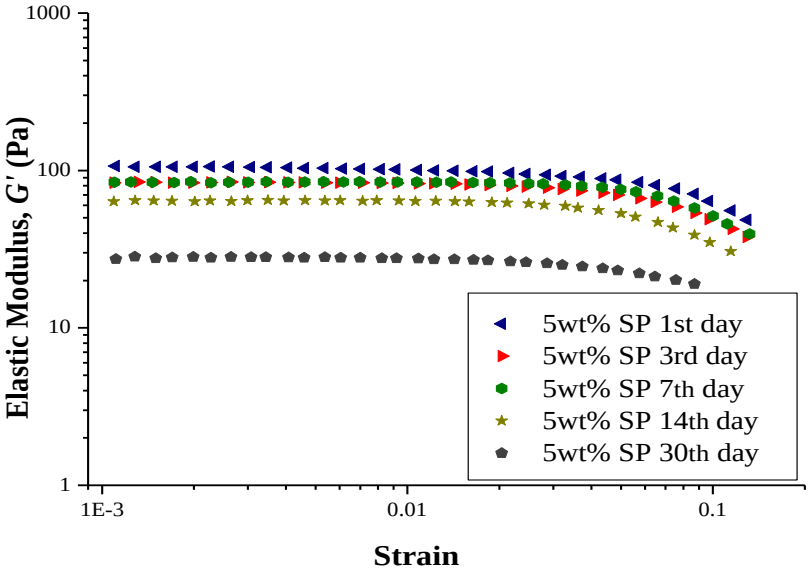


Figure 3.34: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of lavender emulsion stabilized by sucrose stearate after 30 days.

(a)



(b)



(c)

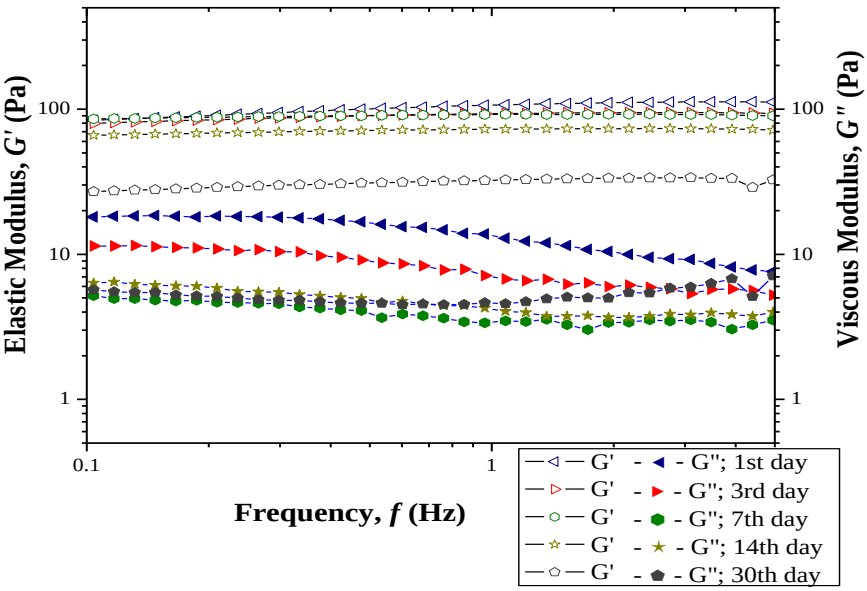
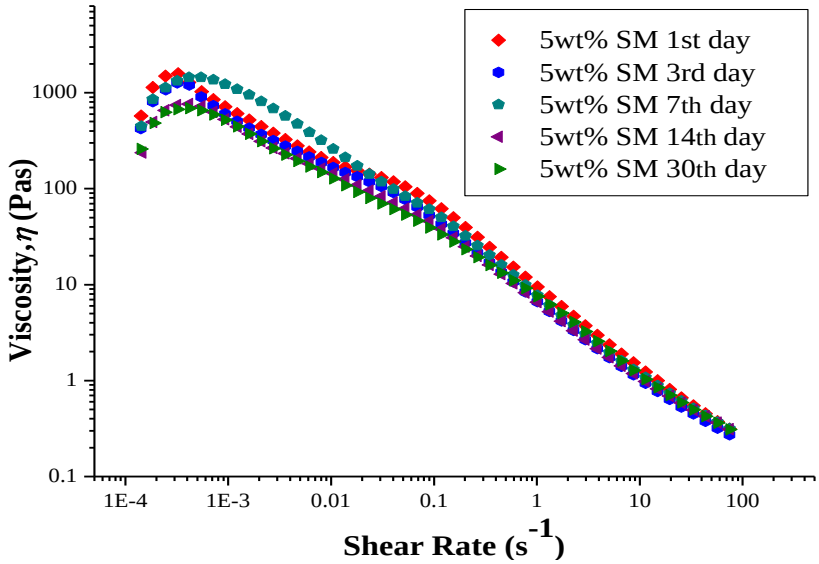
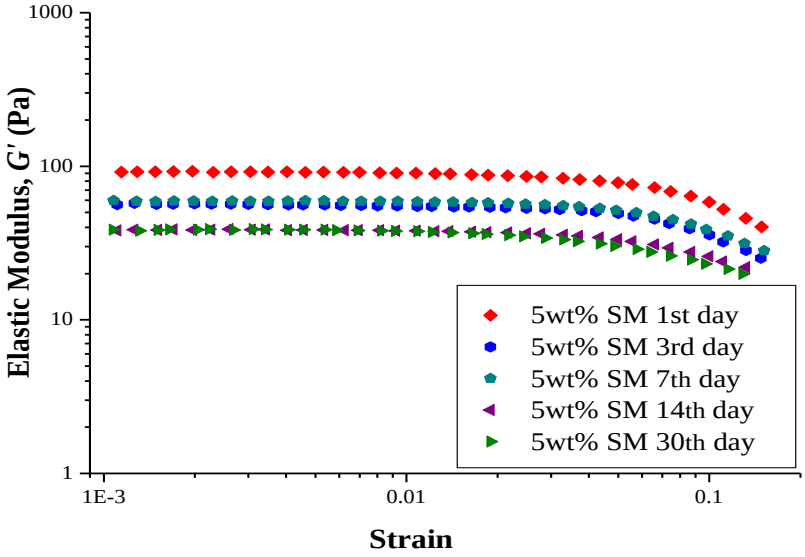


Figure 3.35: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of lavender emulsion stabilized by sucrose palmitate after 30 days.

(a)



(b)



(c)

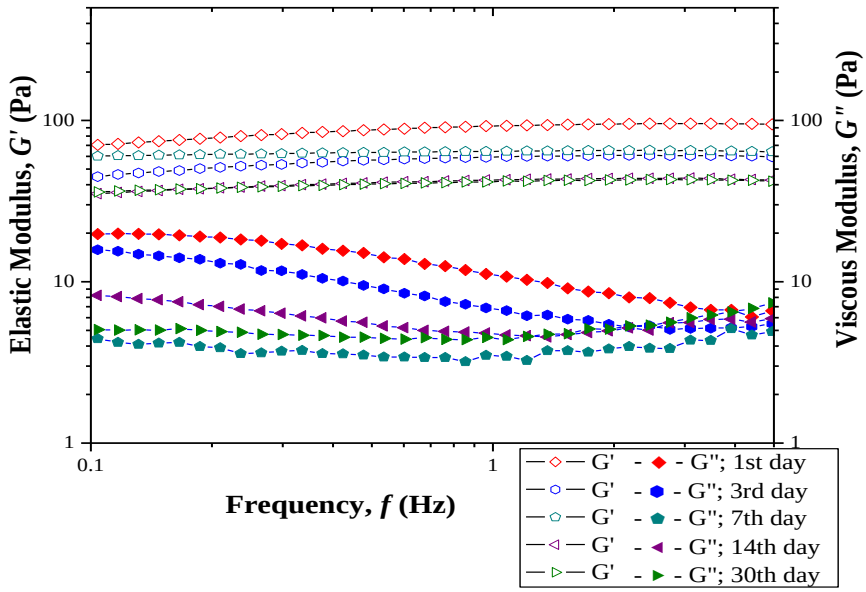
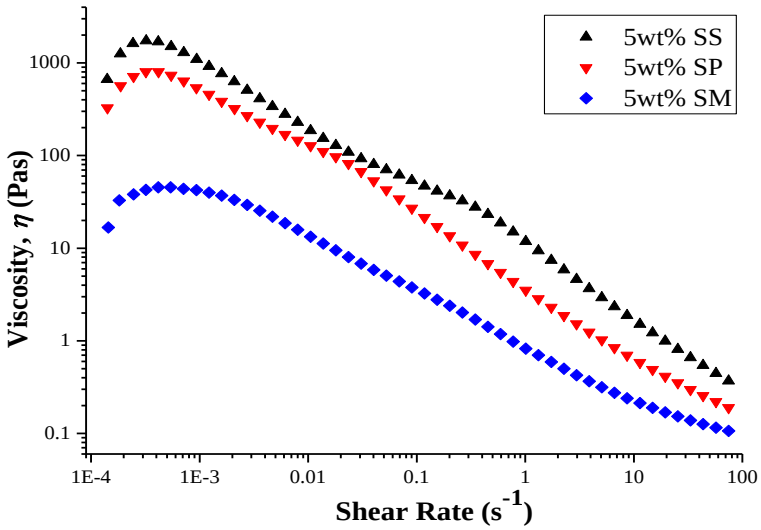


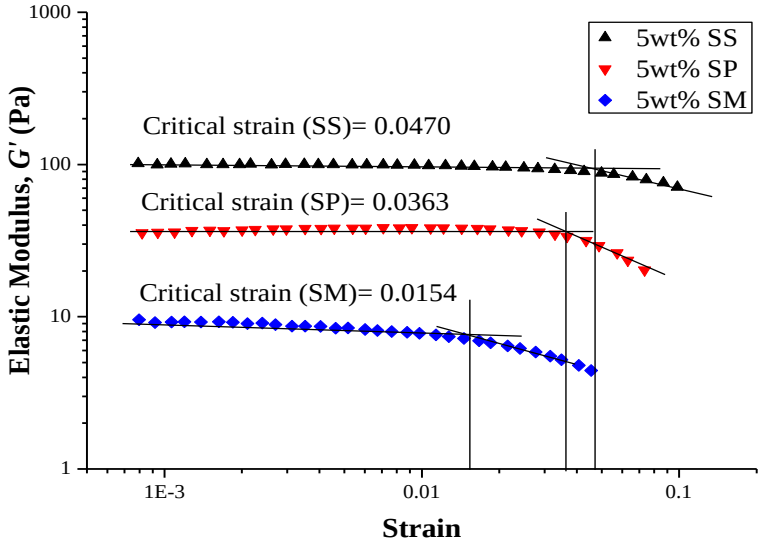
Figure 3.36: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of lavender emulsion stabilized by sucrose myristate after 30 days.

3.6.3 Rheological Analysis of Tea Tree Essential Oil Emulsions

(a)



(b)



(c)

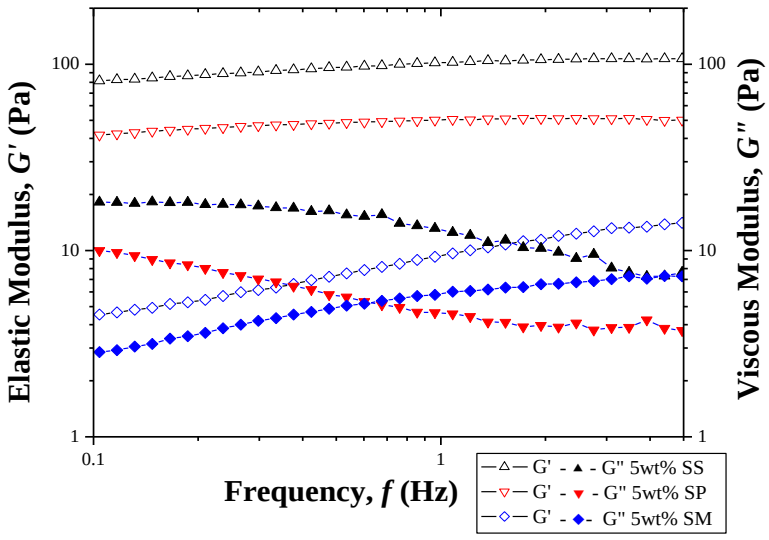


Figure 3.37: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of tea tree emulsion stabilized by glycolipid surfactants on first day of observation.

Tea tree oil emulsions showed significant major differences between essential oil emulsions by presenting data which do not obey the presumably similar trend of rheological profiles. Tea tree oil emulsions stabilized by SM showed critically low viscosities as compared to both SS and SP emulsions. Phenomenon observed was attributed to the formations and arrangement of tea tree oil chemical structure with co-surfactant of ceteareth-20 during the phase inversion of emulsions. The ceteareth-20 acting as a thickener in the system does not take part in the entanglement of the droplets in the dispersed phase causing relatively low viscosity of SM emulsions behaving close to Newtonian fluid flow.

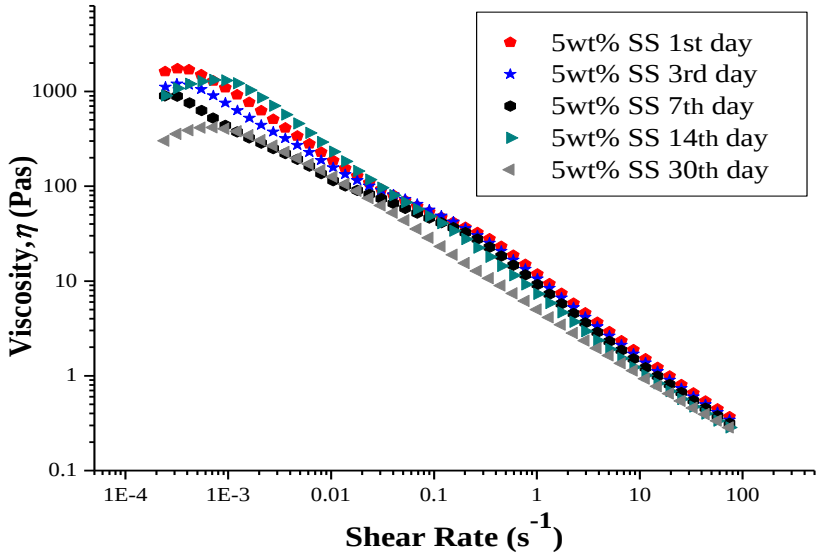
Overall this affected the stability of emulsions formed, as thickener function in forming weak gel system in the continuous phase generating pronounced yield stress preventing movements of droplet from sliding past each other until a sufficient force was applied [184]. Without this function, emulsions generated were weak, easily deformed and unsustainable for a long period. On the other hand, SS and SP emulsions with greater viscosity were predicted to have higher storage stability than SM emulsions as high interfacial viscosity with low shear thinning rates enhanced stability of emulsions by delaying droplet coalescence causing higher resistance towards deformations [185].

The amplitude dependent oscillations test conducted gives an indication that SS emulsions possessing the highest *G'* and longest LVR to be the strongest and resilience towards deformations followed by SP emulsions. SM emulsion was weakest among all. Figure 3.37 (b), frequency dependence of SM emulsions illustrated increasing parallel *G'* and *G"* moduli over sheared frequency denote emulsions system with moderate structural strength and little network structure [186]. Figure 3.37 (a) exhibits a weak and brittle emulsion system of SM emulsion recording low *G'* and critical strain prone to destabilization of emulsions.

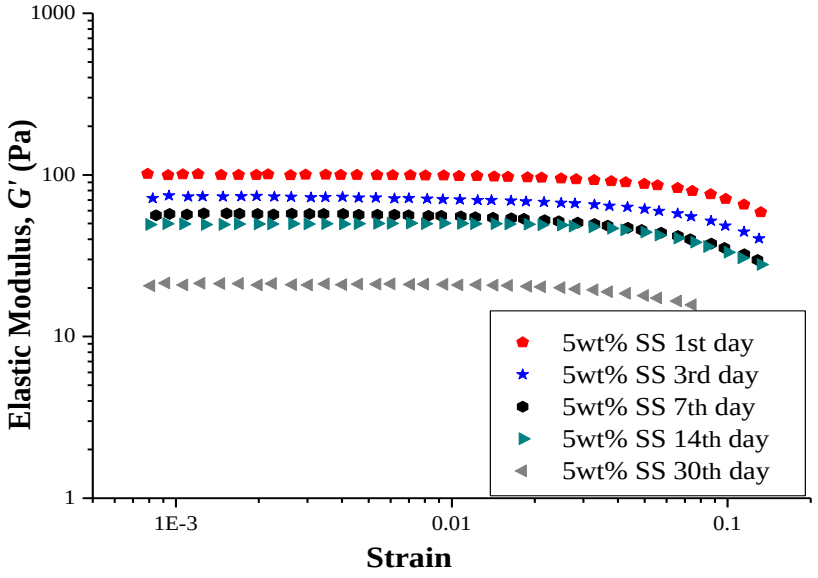
Consecutive study for both SS and SP emulsions yield similar trend of rheological profile with declining stability characteristic depicted in Figure 3.38 and Figure 3.39. Viscosity profile portrayed small degree of decrease in viscosity as a function of shear rate for both SP and SM. Decrease in yield stress of the system reduces the stress necessary to move droplet, since yield stress is the stress required to overcome inter-particle attractive forces to initiate flow [187]. These imply minor loss in cohesive forces and loss of structural integrity of emulsions formed [179, 185]. Acting coherently with frequency sweep denotes decreasing *G'* and *G"* moduli in magnitude with increase storage duration until 14^{th} days of storage. Rapid drop of yield stress and *G'* of emulsions systems on 30^{th} day imply onset of structural disintegration of emulsion system with increase flow ability of emulsion susceptible to phase separation exceeding 30 days of storage. Nonetheless, SP and SS emulsions were still deemed stable compared to SM emulsions.

In the exception of tea tree oil emulsion stabilized by SM glycolipid surfactant (Figure 3.40), sudden increase of storage energy at 30^{th} day was recorded despite initial decreasing trend of rheological profiles. Evolving droplet size leads to formation of droplets with greater size through coalescence slightly increases the viscosity SM emulsions, with overall increases of the storage energy of emulsions at 30^{th} days of observation. Frequency sweep responded in an increasing gap size with increasing magnitude of *G'* and *G"* moduli specifying stronger structural network development in SM emulsion with increasing storage period [180]. Stability of emulsion was enhanced at the end of test, however due to the low storage energy magnitude SM emulsion they was categorized as least stabilized emulsion system compared to SS and SP emulsion system. Higher storage energy, *G'* implies higher stability of emulsion system formed [188].

(a)



(b)



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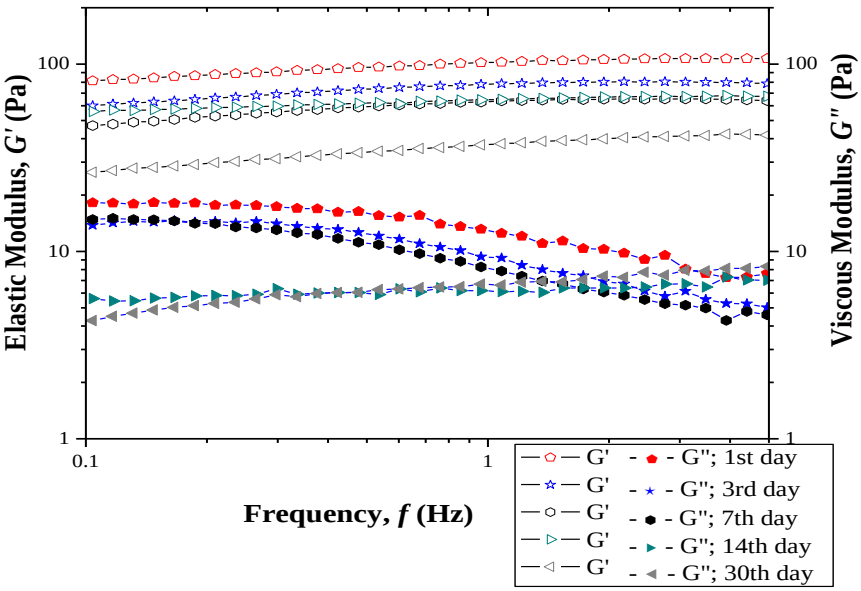
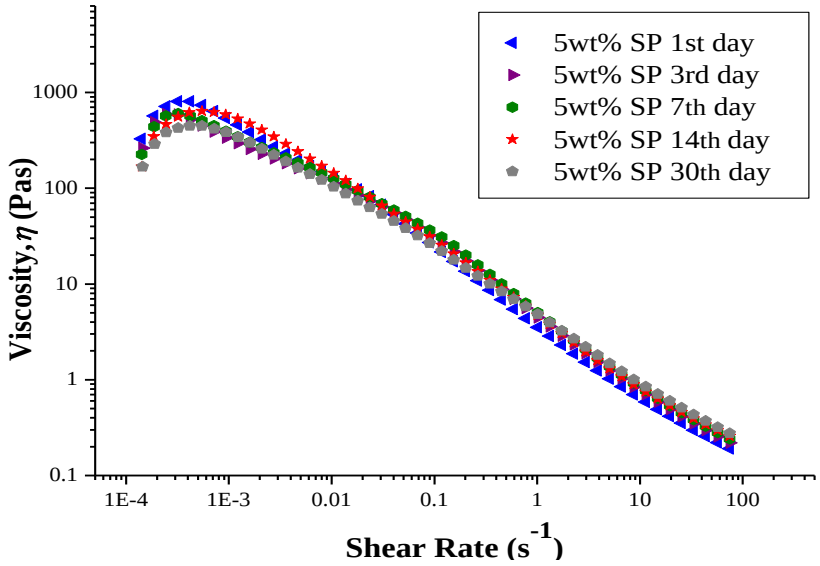
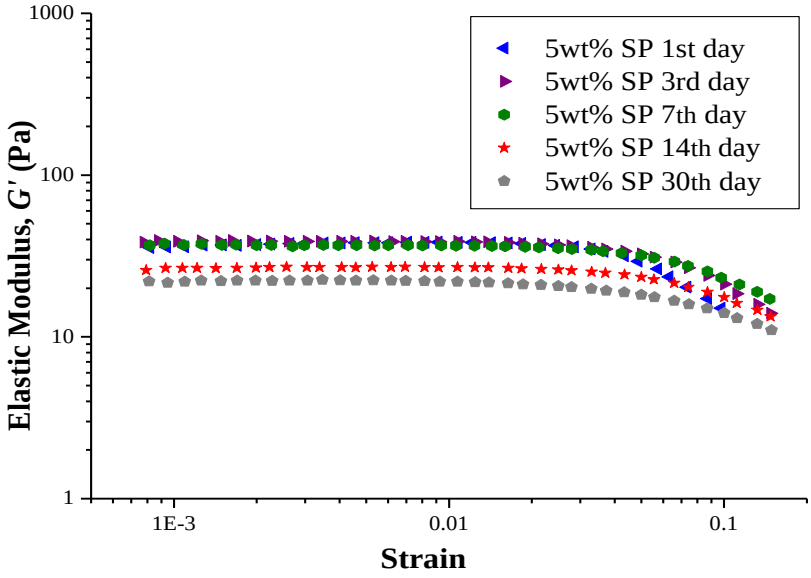


Figure 3.38: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of tea tree emulsion stabilized by sucrose stearate after 30 days.

(a)



(b)



(c)

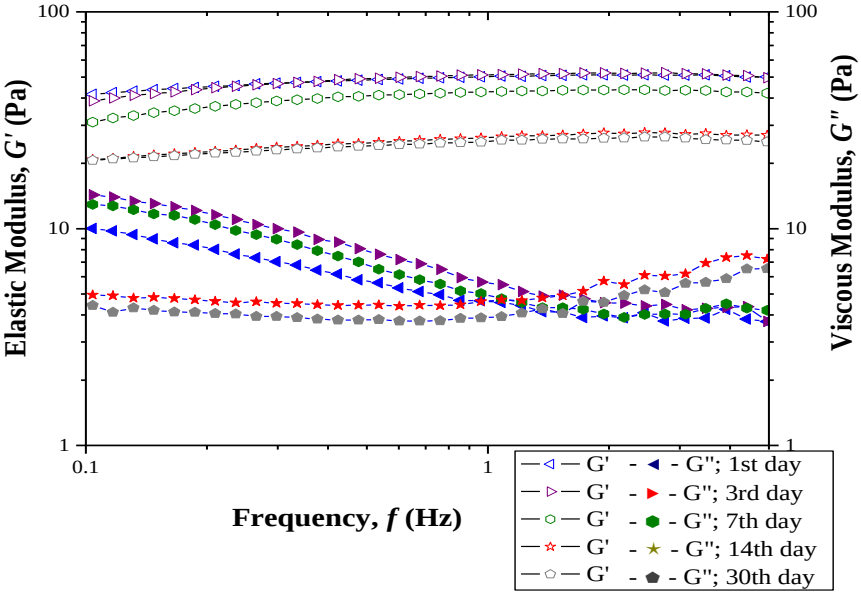
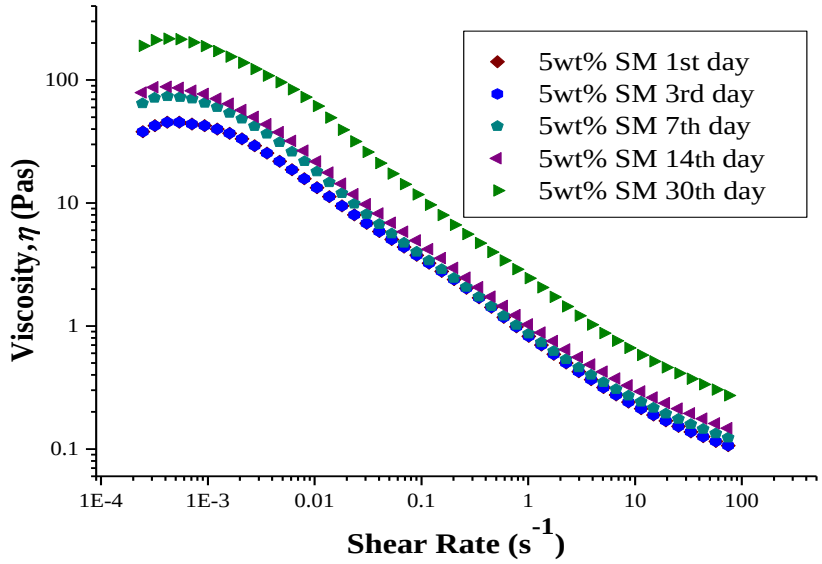
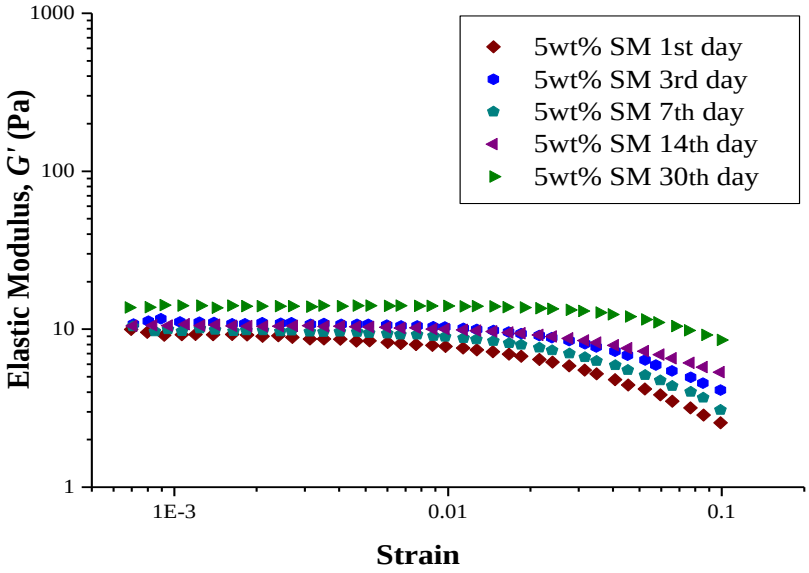


Figure 3.39: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of tea tree emulsion stabilized by sucrose palmitate after 30 days.

(a)



(b)



(c)

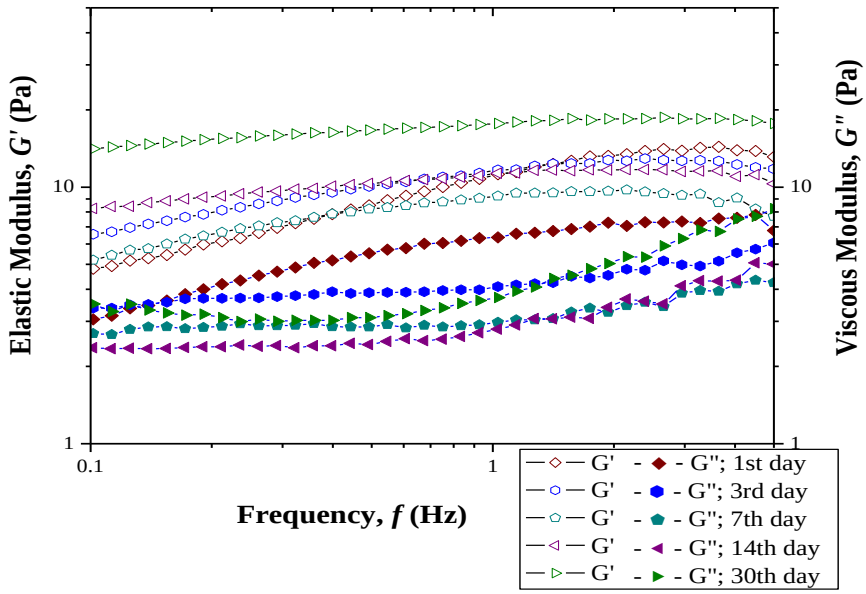


Figure 3.40: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of tea tree emulsion stabilized by sucrose myristate after 30 days.

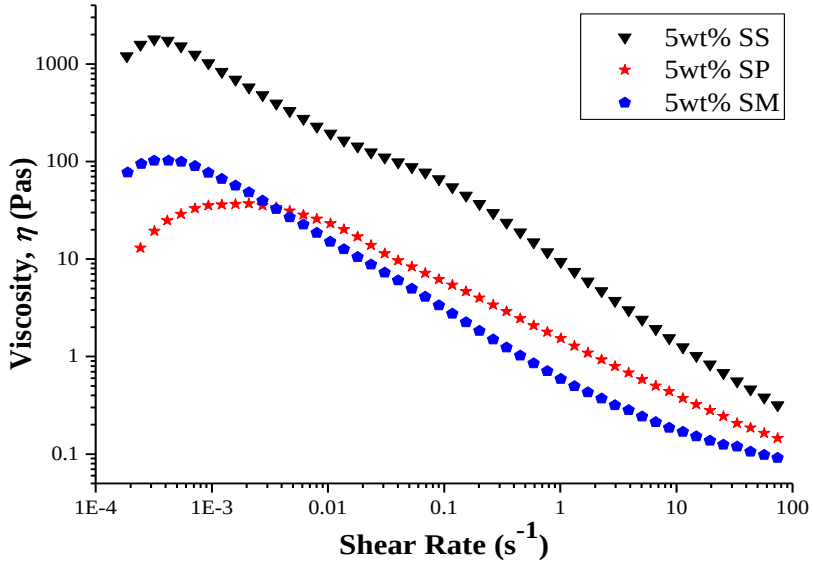
3.6.4 Rheological Analysis of Citronella Essential Oil Emulsions

Citronella emulsions present similar traits of emulsion characteristic resembling those of tea tree oil emulsions with SS emulsion having the strongest and most versatile emulsions characteristic with long LVR and high *G'* magnitude and weak emulsion characteristic with low *G'* and short LVR of SP and SM emulsions. The differences of storage energy between citronella oil emulsions system were significant with SS emulsions recorded 10 times higher than SP and SM emulsions systems. The phenomenon observed was greatly attributed to the dispersions of ceteareth-20 as thickening agent in the SS emulsions which increases the structural integrity of the emulsions which increases the viscosity and storage energy of the emulsions system.

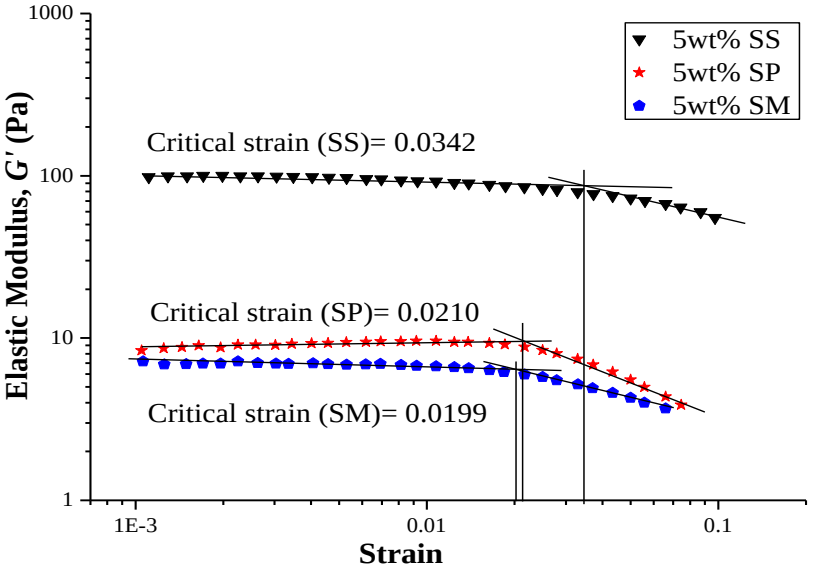
On the other hand, having ceteareth-20 taking part in the formations of droplet for both SP and SM emulsions resulted in minimal structural integrity in the emulsions formed which causes significantly low storage energy as displayed in Figure 3.41 (b). Although, SM emulsions displayed higher yield stress than SP emulsions, the interfacial rheology of SP emulsions reduces the rate of film thinning presenting lower flow-ability of interfacial element indicating higher viscosity at the course of shear. This reduces the rate of shear thinning and rate of coalescence of SP emulsions [173, 185, 189]. Due to these factors SP emulsions demonstrate higher *G'* and critical strain than SM.

Citronella emulsion stabilized by SS glycolipid emulsion (Figure 3.42) display decreasing trend of viscosity and *G'* marking the onset of dissociation of linked internal network structure of emulsion which reduces the ability to withstand deformations [186]. Inter-particle cohesive energy decline with reduced *G'* according to equation 8 inducing destabilization by enabling rapid droplet coalescence [189].

(a)



(b)



(c)

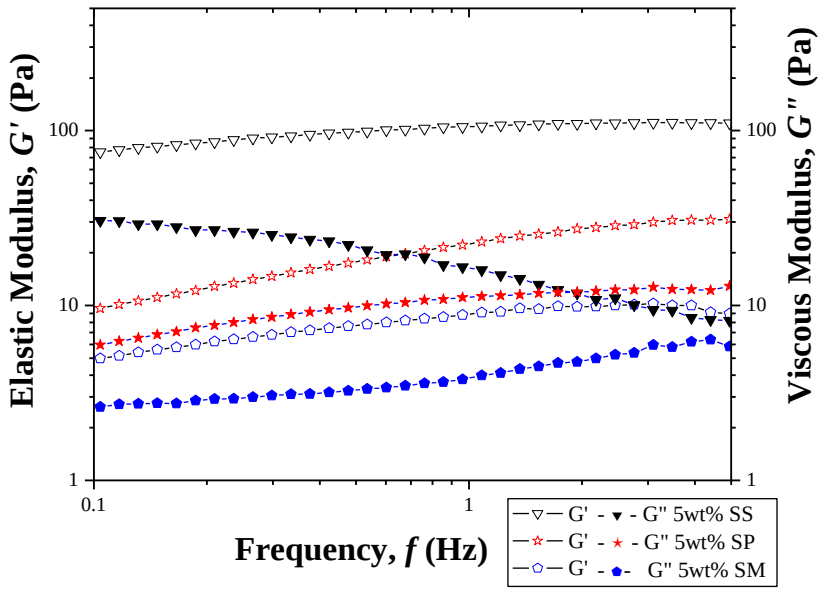
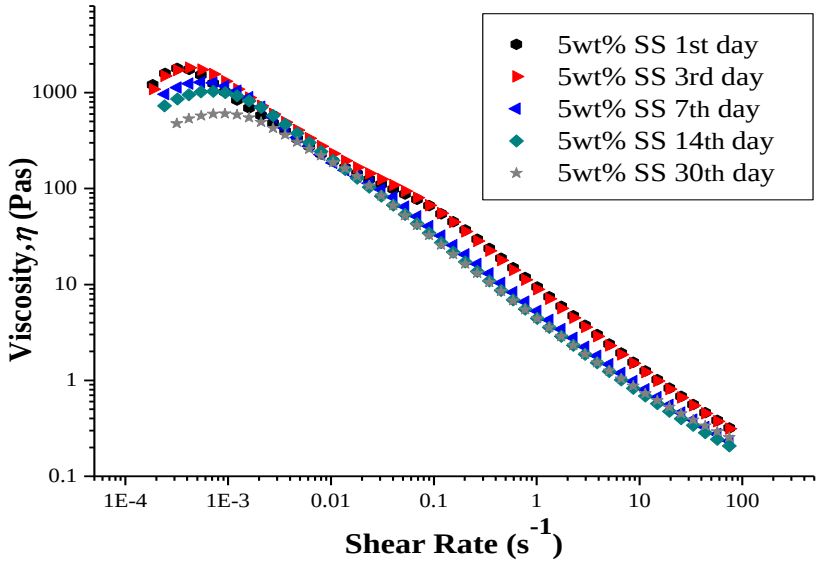


Figure 3.41: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of citronella emulsion stabilized by glycolipid surfactants on first day of observation.

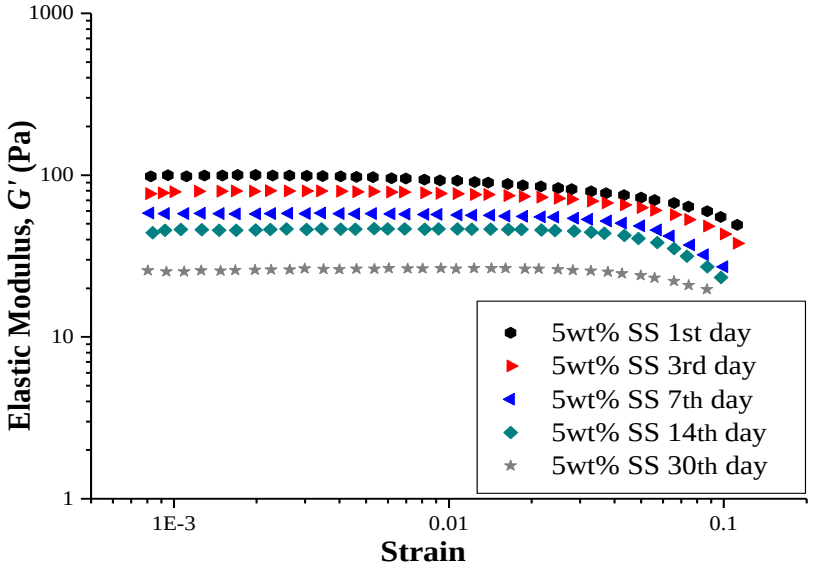
Frequency dependent oscillation shearing of SS emulsions displays decreasing energy of *G'* and *G"* moduli indicated by gap narrowing of frequency profile implying loss of network structure withholding droplets [187]. This indicates that the solid like behaviour of emulsions decreases with increasing trend of fluidity. In spite of that, SS emulsion acquiring higher storage energy was categorized as having higher stability over both SP and SM emulsions [175].

Over the period of 30 days, SP and SM emulsions show gradual increase of storage energy consistent with increase of mean droplet size of emulsions and lowering of PDI. Bigger droplet formation with lower PDI promotes packing of emulsion droplet resisting deformation hence attaining higher storage energy and enhanced viscosity of emulsion over the storage period [182, 184]. Frequency sweep demonstrates increasing trend of network structure illustrating less dependent widening gap of *G'* and *G"* moduli in the frequency measured [186]. SP and SM emulsions were stated to display kinetic stability by retaining high emulsion fractions over storage period even though they possessed low storage energy and viscosity.

(a)



(b)



(c)

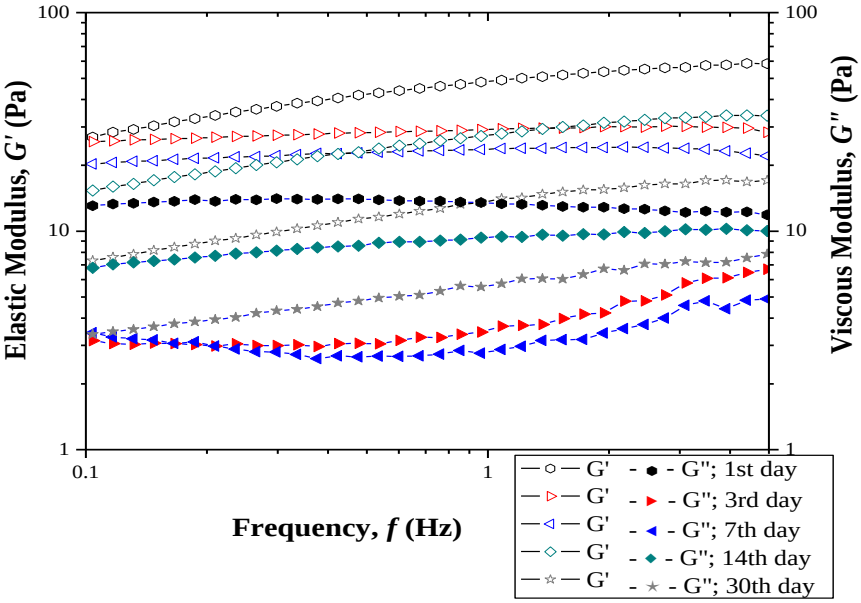
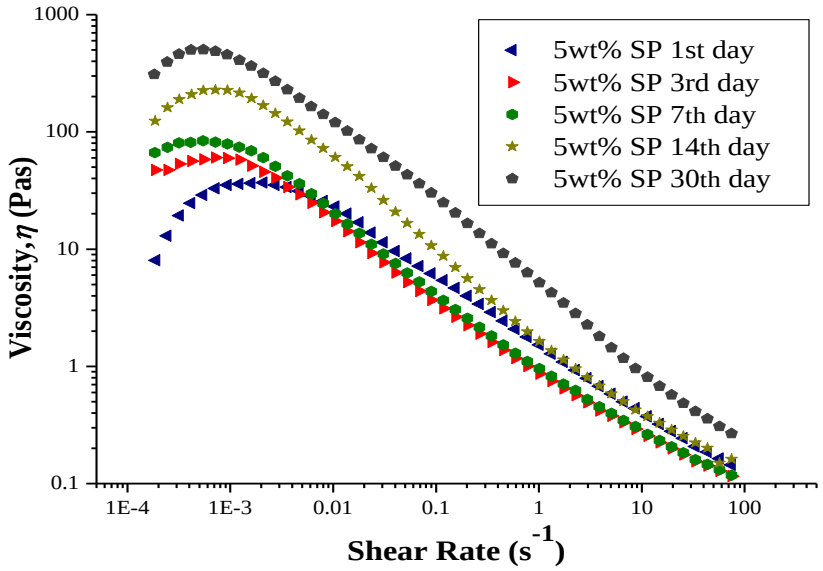
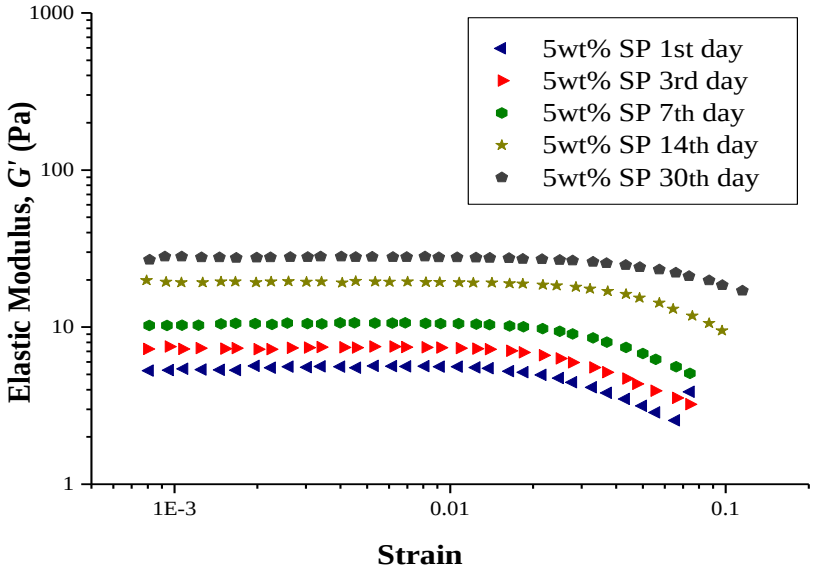


Figure 3.42: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of citronella emulsion stabilized by sucrose stearate after 30 days.

(a)



(b)



(c)

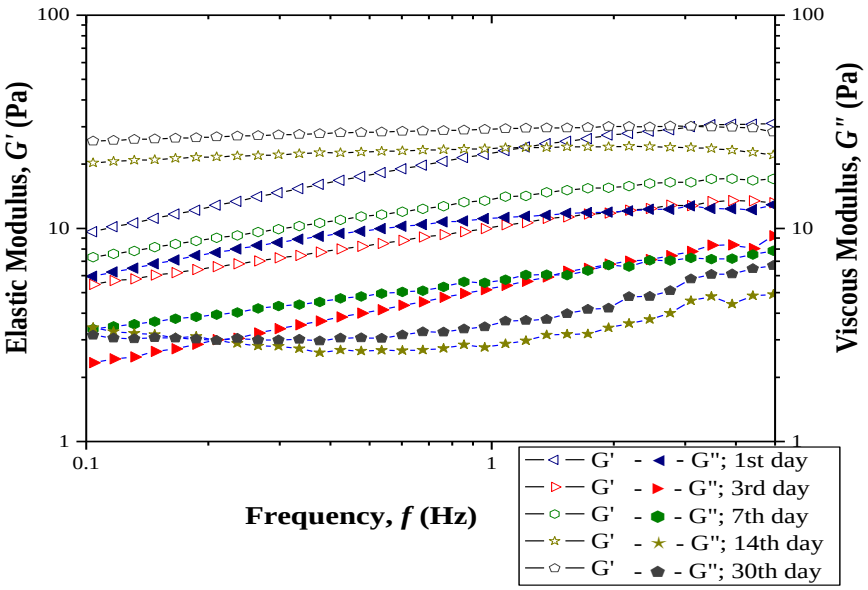
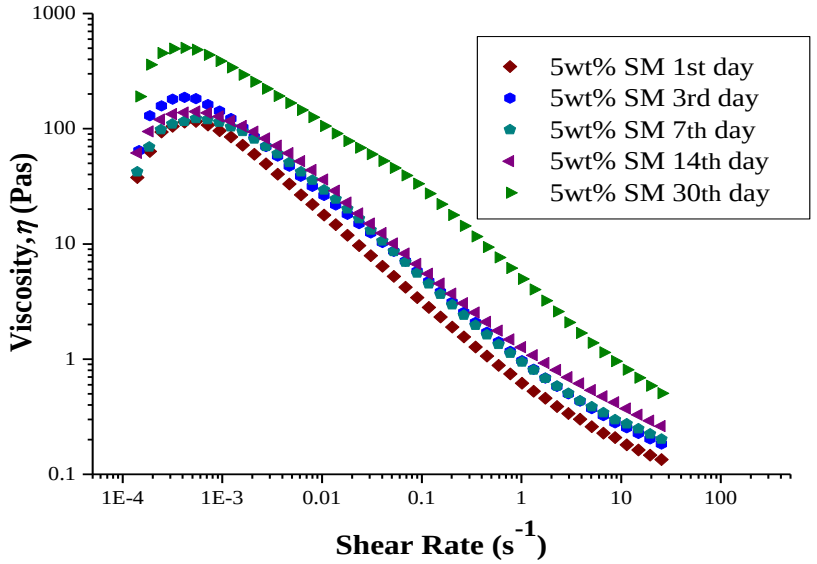
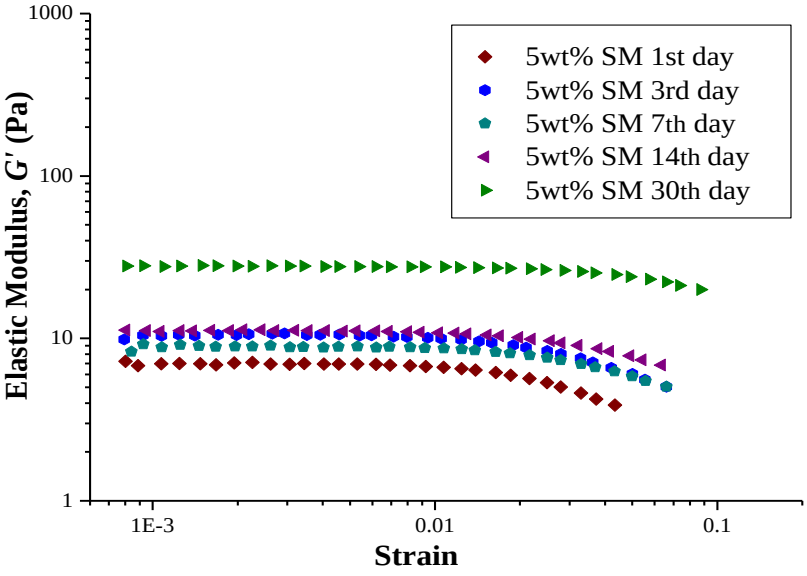


Figure 3.43: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of citronella emulsion stabilized by sucrose palmitate after 30 days.

(a)



(b)



(c)

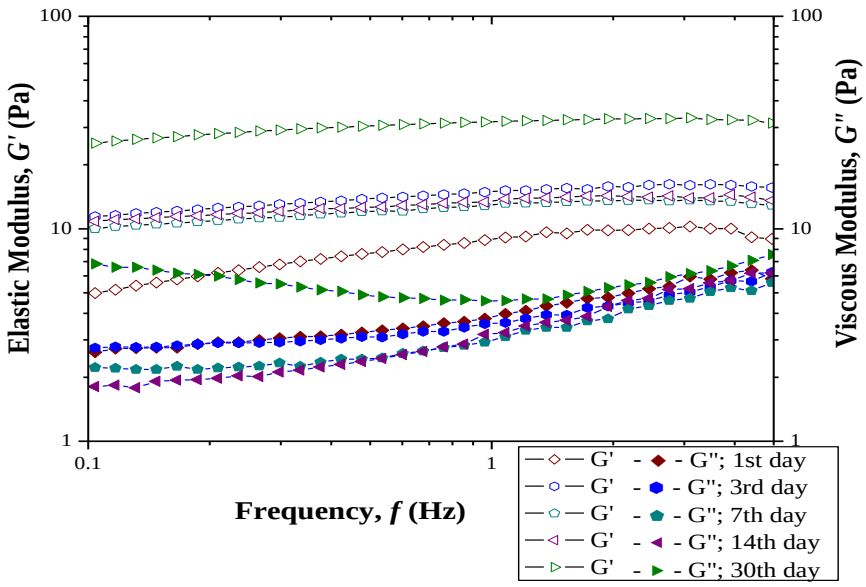


Figure 3.44: Rheological analysis of (a) viscosity as a function of shear rate, (b) amplitude sweep and (c) frequency sweep of citronella emulsion stabilized by sucrose myristate after 30 days.

CHAPTER 4: CONCLUSIONS

Emulsions containing high concentration of essential oils were formulated as an effort to extend its potential applications in cosmetic industry. Formations of highly concentrated essential oil emulsions proved viable with aid of co-surfactants namely Triton^{TM} X-100, Triton^{TM} X-45 and ceteareth-20. The formula of thyme essential oil was determined to be 5 wt% of glycolipid surfactant, 12.5 wt% of thyme oil in total volume of emulsion, o:w ratio of 1:1, 0.007 wt% of Triton^{TM} X-100 and 0.2 wt% of ceteareth-20. Lavender essential oil emulsions formula was determined to be 5 wt% of glycolipid surfactant, 12.5 wt% lavender oil, o:w = 1:1, 0.010 wt% Triton^{TM} X-45, 0.002 wt% Triton^{TM} X-100, and 0.7 wt% ceteareth-20. The most favourable emulsion determined for tea tree oil was 12.5 wt% tea tree oil, o:w = 1:1, 0.009 wt% Triton^{TM} X-45, 0.004 wt% Triton^{TM} X-100 and 0.2 wt% ceteareth-20. Formula of citronella emulsions was determined to be 12.5 wt% citronella oil, o:w = 1:1, 0.002 wt% Triton^{TM} X-45, 0.008 wt% Triton^{TM} X-100 and 0.006 wt% ceteareth-20.

We have found that all emulsions prepared in this work possess a long shelf life, resisting changes in the internal structure giving a potentially stable emulsion system over 30 days of storage duration. Thyme and lavender essential oil emulsions have higher stability over tea tree oil and citronella emulsions retaining high storage energy and high viscosity of emulsions fractions. In comparison, citronella emulsion present weaker emulsion characteristic for emulsion system stabilized by sucrose palmitate and sucrose myristate due to the interactions between co-surfactant in emulsion formation and participation of the co-surfactant in emulsion droplet resulting in high fluidity low stability emulsions. The properties of emulsion formed using the same formulation varied significantly with hydrocarbon chain length of glycolipid surfactant. Interaction of essential oil's composition with co-surfactant participating in the formation of emulsions greatly influence emulsion droplet formation.

Flow characteristic of emulsion studied using rheology profiles at constant temperature of 30.0±0.1 °C, imply that essential oil emulsions always exhibit shear thinning behaviour. Essential oil emulsions also implies solid like behaviour with storage modulus (*G'*) dominant over loss modulus (*G"*) over the measured frequency sweep carried out. The emulsions tested maintain its viscoelasticity even at low viscosity.

We have successfully produced emulsions containing high concentration of essentials oil which meets the current trend of consumer demands in reducing synthetic materials such as fragrances and preservatives in cosmetic products. Essential oil which possessed the properties of natural preservatives and delightful odour served the best combination in providing alternative in replacing the used of synthetic materials in creams and cosmetic products.

Thyme and lavender oil emulsions stabilized by sucrose palmitate give the widest applications of use as these emulsions possessed sufficiently high storage energy which indicates long shelf-life and long LVR which opposes deformation upon shearing. As for tea tree and citronella oil emulsions, sucrose stearate emulsion present the most stable system with the highest storage energy and longest LVR region. To serve the new trends of cosmetic demand, a wide range of product is needed to be more competitive in the industries and for different applications. Therefore, prototype of these emulsions can be produced by referring to the determined formulae respectively and can readily be used in cosmetic and pharmaceutical industries.

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